MICROPLASTIC POLLUTION IN INDIANA'S WHITE RIVER: AN EXPLORATORY STUDY

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LINDSAY L. HYLTON

DR. JESSIQUE GHEZZI – ADVISOR

BALL STATE UNIVERSITY

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ABSTRACT

THESIS: Microplastic Pollution in Indiana's White River: An exploratory study

STUDENT: Lindsay Hylton

DEGREE: Master of Science

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Plastic material is now a ubiquitous source of aquatic pollution, most of which originates from sources on land, including surface waters. Microplastics- tiny plastic pieces that are often not visible to the naked eye, are a recent growing environmental concern in both marine and freshwater ecosystems. While many studies have proven the abundance and danger of microplastics in the world's oceans, far less research has been done on their presence and impact in freshwater ecosystems. This exploratory quantitative study aims to build on the findings of recent freshwater microplastic studies by reporting on the abundance and types of microplastic pollution found in the West Fork of Indiana's White River. Fifteen surface water samples were taken from three bridge sites along the river over a four-month period (August-November 2015). Samples were collected with a custom adapted net and analyzed using NOAA's recommended laboratory methods for the analysis of microplastics in the marine environment. Analysis revealed a variety of microplastic particles at all three sampling sites, to varying extents, with synthetic fibers being the predominant plastic type collected. A total of 145.5 plastic pieces were collected from the White River over the duration of this study, from all 15 samples (which were averaged with duplicates). The average microplastic concentration in the White River, based on the average concentrations of the three sites sampled, was 0.71 items/m^3. These findings help to

fill the large knowledge gap in microplastic research on freshwater, especially fluvial, environments and guide researchers in better understanding the extent to which these synthetic particles are polluting U.S. surface waters as a whole.

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INTRODUCTION

Plastic - the lightweight, inexpensive, and durable material that we all encounter on a daily basis has become an indispensable part of modern life. It has also become one of the most pervasive and abundant sources of marine pollution in existence (Moore 2008; Thompson et al. 2009; Wright et al. 2013). Its unique properties and uses have increased its global production to nearly 355 million tons as of 2015 (Plastics Europe 2016). Accordingly, plastic material now also makes up 60-80% of marine debris, through accidental release and careless discarding by its many users (Eriksen et al. 2013; Wright et al. 2013). The proliferation and wasteful use of disposable plastic items has allowed plastic pollution to become a major global environmental problem. The recent discovery of microplastic particles in remote aquatic environments, and in surprisingly high abundance in freshwater ecosystems like the Laurentian Great Lakes, has resulted in growing scientific and public concern.

The effects of large pieces of plastic debris, called *macroplastics*, on the marine environment have been the focus of many scientific studies, due to their visibility on beaches and obvious harm to wildlife. *Microplastics*, however, have only been studied on their own in much more recent years. These tiny plastic spheres, fragments, and fibers, often not visible to the naked eye, are a growing environmental concern for multiple reasons. First, their large surface areas serve as absorptive surfaces for hydrophobic persistent organic pollutants (POPs) that occur in water (Andrady 2011). Second, their chemical composition makes them capable of leaching their toxic plasticizers. And third, their size permits ingestion by thousands of species of organisms across the food web. This leads to the possibility of bioaccumulation of the aforementioned pollutants, in addition to their physical accumulation, within various wildlife (Cole et al. 2011; Wright et al. 2013).

While many studies have now proven the abundance of microplastic pollution in the world's oceans, far less research has been done on their presence or impacts in freshwater ecosystems, especially within rivers. We know now that plastic pieces are reaching remote and unexpected parts of the planet and it is critical to understand the role that rivers are playing in this equation (Free et al. 2014; Woodall et al. 2014). Therefore, it is the goal of this exploratory quantitative study to build on the findings of recent freshwater microplastic studies by investigating the abundance and types of microplastic pollution in the West Fork White River in Indiana. The results may also provide a clearer picture of how plastic concentrations differ spatially between different locations along a river with varying watershed characteristics.

The research aims to answer the following questions:

- What is the plastic abundance, or concentration, at three different locations on the West Fork White River?
- How will concentrations differ between site locations with varying subwatershed characteristics and populations? Will the Muncie Water Pollution Control Facility cause higher plastic concentrations at Site 2 (just downstream of the facility) than those of Site 1 (just upstream of the facility)?
- What types of microplastics are occurring in the White River? Is there an abundance of "microbeads" from personal care products, as seen in the Great Lakes (Erikson et al. 2013)?

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LITERATURE REVIEW

Introduction to microplastics

Definition

Microplastics are fragments and primary-sourced plastics smaller than 5 mm in size (Arthur et al. 2009; Ivar do Sul & Costa 2014). Microplastics can be classified into two different categories, primary and secondary, based on their source. Primary microplastics are those manufactured to be microscopic in size, primarily for industrial and domestic use, and secondary microplastics occur due to the fragmentation process of larger plastic debris breaking down naturally in the environment.

Primary Microplastic Sources

Primary microplastics are created in multiple forms for a variety of purposes. Of most recent and public concern are those being found in exfoliating facial cleansers, body washes, hand soaps, and even toothpastes. Certain brands of these products began adding polyethylene pieces, or "scrubbers," to replace the natural exfoliates (such as pumice or apricot husks) once used by manufacturers (Fendall & Sewell 2009; Gregory 1996; Zitko & Hanlon, 1991). After the product's use, these plastic pieces are washed down the drain with the product and end up in city wastewater systems. Here, they can bypass removal by the initial coarse treatment screens, making their way into the final effluent and sewage sludge (Cole et al. 2011; Derraik 2002; Fendall & Sewell 2009; Vesilend 2003). Numerous studies are showing these particles are found in both marine and freshwater bodies. Rochman et al. conservatively estimates that approximately 8 trillion microbeads per day are emitted into U.S. aquatic habitats. "Studies report a range of 0-7 microbeads L^{-1} of final effluent, which is often discharged directly to

aquatic habitats" (2015, p.10759). Further, they explain that assuming 99% of these particles are removed during the treatment plant's sedimentation process, the remaining 800 trillion microbeads will settle into sludge, which can be spread over land as low-grade fertilizer. Precipitation and irrigation will allow these microbeads to eventually reach waterways through runoff (Rochman et al. 2015).

This is cause for concern as it represents an unnecessary contribution of micro-litter of which many consumers are unaware. As the issue has become more publicized by the media, especially with the recent discovery of microbead abundance in the Great Lakes, a handful of large companies have pledged to remove the plastic scrubbers from their "rinse-off personal care products" (Rochman et al. 2015). President Obama ensured that this will occur by signing a bill, the Microbead-Free Waters Act of 2015, which will require manufacturers to eliminate the pollutant from their product by 2017.

Other sources of primary microplastics are less well-known by the general public but have existed for quite some time. Microplastics produced for use in air-blasting technology help to remove rust and paint from machinery, boat hulls, and engines (Browne et al. 2007; Cole et al. 2011). Additionally, the virgin resin pellets used to create numerous plastic products could also be placed into this category, although they could be considered larger than the allowable size limit for microplastic pollution. The pellets regularly enter the oceans through loss during ocean transport and through run-off from processing facilities (Cole et al. 2011; Gregory 1996).

Secondary Microplastic Sources

Eighty percent of the plastic debris found in the marine environment comes from land-based sources, with highly populated or industrialized areas serving as major contributors, due to

littering and solid waste disposal (Derraik 2002; Li et al. 2016). Coastal recreational activities, raw manufacturing materials, wastewater effluent, and refuse site leachate are some of the known sources (Browne et al. 2010; Li et al. 2016). River systems, wastewater treatment works, and even extreme weather events transfer these plastics to the sea (Barnes et al. 2009; Browne et al. 2010; Cole et al. 2011).

The remaining twenty percent of marine litter, according to Li et al., can be attributed to ocean-based sources, with the commercial fishing industry as the prime contributor (2016). Good et al. (2009) estimates that approximately 705,479 tons of discarded fishing gear is lost into the ocean annually (Li et al. 2016). In addition to becoming microplastics, monofilament lines and nylon netting, when discarded, are also known to float at a certain depth and entangle organisms in what's known as "ghost fishing" (Li et al. 2016; Lozano & Mouat 2009).

Secondary microplastic debris can be derived from various classes of plastics that come from land-based sources, primarily plastic packaging (including disposable single-use items), as well as fishing industry litter (Andrady 2011). Consumer packaging material and disposable items make up almost a third of the plastic resin production and therefore have a high likelihood of becoming ocean litter. According to Andrady, "Several broad classes of plastics are used in packaging: Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Poly(ethylene terephthalate) (PET); and Poly(vinyl chloride) (PVC)" (2011, p. 1597). Other classes of plastics that are commonly found in the marine environment are Foamed Polystyrene, Nylon (PA), and Cellulose Acetate (CA) (Andrady 2011).

Much of this production ends up on beaches as litter, and is subject to the natural weathering process (Andrady 2011). According to Li et al., beaches are the ideal setting for plastic fragmentation due to higher oxygen availability and high levels of sunlight. Once in the

water or on a beach, the fragmentation process of macroplastic litter will begin by either chemical or mechanical weathering, or by photodegradation caused by sunlight, all of which make the plastic weak and brittle (2016). Li et al. explain that, "The ultraviolet radiation in sunlight causes oxidation of the polymer matrix, resulting in chemical bond breakage" (2016; Barnes et al. 2009). Abrasion by sand or wave action helps to further break the plastic into tiny fragments, which can become increasingly smaller (Andrady 2011; Barnes et al. 2009; Ivar do Sul & Costa 2014). Thermal and/or chemical degradation can also make plastic material susceptible to biodegradation by microbes (Andrady 2011; Ivar do Sul & Costa 2013; Shah et al. 2008).

Differences in the generation of secondary source microplastics between marine and freshwater environments are still unknown. The weathering and fragmentation rates of plastics in marine systems, which have been studied more extensively, are also not known (Eerkes-Medrano et al. 2015). While plastics in marine and freshwater systems both experience physical and chemical degradation, forces such as wave action and storms may cause differing levels of physical weathering on marine plastics (Andrady 2011; Eerkes-Medrano 2015).

Synthetic Fibers and Microbeads from Wastewater Effluent

Another source of microplastic pollution that scientists are now paying more attention to are tiny synthetic fibers that most people encounter every day. According to Habib, Locke, and Cannone, synthetic fibers were first utilized by the textile industry over 50 years ago, in order to supplement natural fibers such as wool, cotton, and linen (1998). The authors explain that:

> Synthetic fibers such as *Nylon©, Orlon©, Dacron©, and Spandex©* are now widely used in clothing, carpets, upholstery and other such materials. Laundering synthetic textiles releases fibers into sewage systems and thus into sewage treatment plants. Because

> synthetic fibers are not readily decomposed by aerobic or anaerobic bacteria in sewage treatment plants, they concentrate in sewage sludge and are also discharged in effluents. (Habib et al. 1998, p. 3)

As clothing are continuously washed and dried, worn and broken fibers break loose due to this mechanical action and become suspended in the gray (waste) water. They then go through the wastewater treatment process and often persist as part of the effluent or sludge that is often eventually incorporated into the natural environment in various ways (Habib et al. 1998). Rivers are a likely source of transport for these fibers through the aquatic environment, while the application of digested and dewatered sludge as a low-grade fertilizer, a common agricultural practice, is a another likely source of transport throughout a watershed.

A recent study by Murphy, Ewins, Carbonnier, and Quinn sought to estimate the amount of all types of microplastic pollution leaving a wastewater treatment plant each day. They believe that Wastewater Treatment Works (WwTW) could possibly be a major source of microplastics into the aquatic environment. The use of microbeads in personal care products and the widespread use of synthetic clothing (polyester and nylon) are the reasons for this assumption (Murphy et al. 2016). Their study investigated the effectiveness of the WwTW process in removing microplastic particles from municipal effluent. To do this they sampled throughout the various stages of the treatment process of, “a large secondary WwTW with a population equivalent of 650,000” (Murphy et al. 2016).

Their study found no microbeads in the final effluent, despite recent concern about microbeads passing through the various screens of the treatment process. Since the majority of plastic scrubbers used in personal care products are made of positively buoyant polyethylene, they are likely to sit on the water’s surface and get skimmed off during the grease removal stage.

This was where they found that the majority of microplastics were indeed removed. Ultimately, the authors concluded that despite the efficient rates of microplastic removal by a modern WwTW, their calculations proved that the plant was still releasing 65 million microplastics into the receiving water daily, due to the large volume of effluent being released (Murphy et al. 2016).

Recently, Mason et al. (2016a) sought to conduct a larger-scale wastewater treatment plant effluent study to better understand why the results of Murphy et al. and others (Baltic Marine 2014; Carr et al. 2016; Magnusson & Wahlberg 2014) show a large variation in microplastic counts within the effluent stream. They explain that, "All of these studies indicate that wastewater treatment facilities are quite efficient at removing microplastics from treated wastewater, with calculated removal efficiencies of 95-99%" (Mason et al. 2016a, p. 2). However, due to the large volumes of water being processed at these facilities, considerable amounts of microplastic pollution are leaving in the effluent. Mason et al. aimed to broaden the scope of the aforementioned studies by analyzing effluent from facilities across the United States that vary in size and utilize a variety of treatment technologies (2016a).

After analyzing 90 different effluent samples from 17 different wastewater treatment facilities, they determined that municipal wastewater contains an average of less than one particle per liter of effluent (Mason et al. 2016a). They explain that despite this low number, the estimated daily abundance was much higher, "on the order of tens of thousands to millions of particles per day (i.e., individual facility values ranging from ~5×10^4 to nearly 1.5×10^7 particles per day)" (Mason et al. 2016a, p.4). As was the case in similar studies conducted on WWTPs, fibers were the most prevalent particle found (59%), followed by fragments (33%). Pellets, which would include microbeads from consumer care products, only contributed 1% of

the particles collected. Lastly, smaller plastics (0.125-0.355 mm) were only slightly more abundant than larger plastics (>0.355 mm), with fibers dominating this size class (Mason et al. 2016a).

Microplastics in the marine environment

The majority of the microplastic research to date has looked at the abundance and effects of microplastics on the world's oceans. Only in the last few years have researchers begun exploring the impact of this ubiquitous pollutant in freshwater ecosystems (Eriksen et al. 2013; Wagner et al. 2014; Zbyszewski, Corcoran & Hockin 2014). Due to their light weight, buoyancy, and ability to travel in the water column and surface, we now know that microplastics have reached some very remote places. They have been found in the polar regions, mid-ocean islands, and even the deep sea (Andrady 2011; Barnes et al. 2009; Ivar do Sul et al. 2014; Wagner et al. 2014).

While microplastic pollution is not necessarily a visual nuisance, like large plastics found strewn on beaches, the problem it represents lies in its effect on aquatic wildlife. This debris, which already contains its own chemical additives, can also adsorb organic contaminants from the surrounding water, therefore becoming a vector of various pollutants to the organisms that ingest it (Bakir et al. 2012; Ivar do Sul & Costa 2014; Teuten et al. 2009; Zarfl 2010).

Bioavailability to Marine Organisms

Microplastics' small size, similar to that of sediment, and mobility throughout the water column, classify this debris as bioavailable to a wide range of organisms (Wright et al. 2013). Microbial biofilms play a large role in the location of these particles in the water and in which biota will be likely to access them. Biofilms can develop very quickly on submerged plastic, significantly changing its hydrophobic properties and ability to float on the water's surface

(Lobelle & Cunliffe 2011). Once biofouled, these particles will often sink slowly down to the benthos, where they become available to organisms throughout the water column (Wright et al. 2013).

A study recently conducted by Lobelle and Cunliffe tested polyethylene food bags, submerged in Queen Anne's Battery (Plymouth, UK) to gain more information about how early microbial biofilm formation occurs. They found that after two weeks, the plastic began to float below the water's surface, and by three weeks, the plastic started sinking down below the surface, showing signs of neutral buoyancy (2011). Fouled microplastics can eventually sink all the way to the benthos, similar to high-density plastics, such as PVC (Wright et al. 2013). Once here, they present themselves to benthic suspension feeders and detritivores. Several laboratory studies have shown that marine ciliates (Christaki et al. 1998), echinoderm larvae (Bolton & Havenhand 1998), and zooplankton, have engulfed or ingested microplastics at the water's surface or within the water column (Wright et al. 2013). This is often dependent upon plastic size and coloration.

Physical and Chemical Impacts to Marine Organisms

Multiple laboratory studies have recently proven the detrimental effects that the ingestion of microplastics can have on smaller organisms, including invertebrates. However, a review by Wright et al. stated that the biological impact of this pollution type on marine organisms has received far less attention than studies on entanglement or ingestion of larger macroplastics (2013). Their size, similar to that of sediment and some planktonic organisms, make microplastics bioavailable to a numerous array of organisms. These can include, according to Wright et al., ingestion by, "…low trophic suspension, filter and deposit feeders, detritivores and

planktivores" (2013). In these organisms, accumulation of microplastics can potentially cause internal blockages and abrasions (Wright et al. 2013).

Plastic debris are prone to accumulating persistent organic pollutants (POPs) that occur universally in ocean water at low levels, along with other waterborne pollutants, via partitioning (Andrady 2011). Microplastics are unique and especially susceptible to this adsorption because of their large surface area to volume ratio, and can become contaminated up to six orders of magnitude greater than ambient seawater (Cole et al. 2011; Wright et al. 2013). Mato et al. explains that there are two ways in which chemicals are introduced into marine plastic pellets:

> One is adsorption of hydrophobic chemicals (e.g., PCBs) onto plastic resin pellet surfaces from seawater because of the low polarity (i.e., hydrophobic nature) of the plastic surfaces. Plastic resin pellets may act like hydrophobic adsorbents in the sea. Another plausible mechanism may be plastic additives and related chemicals contained within resins. Various synthetic chemicals are contained in plastic resin pellets as additives, and some are potentially harmful to wildlife. (Mato et al. 2001, p. 318)

Furthermore, when small organisms containing microplastic debris are consumed by higher trophic wildlife, these contaminants have the potential to transfer up the food chain (Betts 2008; Cole et al. 2011; Teuten et al. 2009). According to Cole et al., many POPs are considered toxic and can cause endocrine disruption, mutagenesis/carcinogenesis, and even biomagnification in higher-trophic organisms (2011). Farrell and Nelson published the first study of its type in 2013, proving that "natural" trophic transfer of microplastics can occur. They investigated this transfer from mussels (*Mytilus edulis*) to crabs (*Carcinus maenas*) using 0.5µm fluorescent polystyrene microspheres and found, "microspheres in the stomach, hepatopancreas, ovary and gills of the crabs, in decreasing numbers over the trial period" (2013). They state that

despite the small amount transferred, their study demonstrated the potential for trophic transfer between these two organisms, and that microplastics can translocate to the haemolymph and tissues of the crab (Farrell & Nelson 2013).

Further studies have discovered microplastic ingestion in multiple vertebrate species. A study conducted on the English Channel by Lusher et al. (2013) looked at fish and found that 36.5%, from 10 species that were sampled, contained microplastics, regardless of their habitat (Wright et al. 2013). According to Wright et al., "An average of 1.9 ± 0.1 particles were recovered from those which contained plastic, the main polymers being polyamide and polyester, which are materials commonly used in the fishing industry" (2013, p.489).

A study by Boerger et al. (2010) conducted in the North Pacific Central Gyre, also referred to as the 'Great Pacific Garbage Patch,' found microplastic fragments in one third of the fish that were caught, with the most common species caught (*Myctophum aurolanternatum,* Myctophidae) containing on average six plastic pieces. This fish utilizes a diurnal feeding behavior and preys at night upon plankton near the water's surface (Wright et al. 2013). Boerger et al. believes that the Myctophidae possibly confuse the small plastics for their natural food source, a plankton species that is similar in color to the most commonly ingested plastic colors found in the study (white, clear, and blue) (2010; Wright et al. 2013). It is also a possibility that these fish are consuming plankton that have already consumed microplastic particles themselves, or the Myctophidae may ingest the plastics passively while feeding (Wright et al. 2013). Wright et al. adds that because these fish are commonly preyed upon by tuna, squid, odontoceti whales, seabirds and fur seals, there are numerous pathways for microplastic debris to work its way into the food web by means of fish (2013; Boerger et al. 2010).

Data on the contamination of freshwater wildlife by microplastics is greatly lacking. It is likely, however, that these organisms interact similarly with this pollution as their marine counterparts. Sanchez, Bender, & Porcher aimed to bridge this gap by studying the occurrence of microplastic particles in wild fish from French rivers (2014). They selected the gudgeon (*Gobio gobio*), a sedentary fish that lives in freshwater ecosystems for the entirety of its life cycle, collecting 186 adults from 11 French streams, with varying environmental factors (Sanchez et al. 2014). Their results showed that 12% of the fish collected contained microplastics, and seven out of the eleven stream sites contained contaminated fish. These sites were all considered urban rivers (Sanchez et al. 2014). Additionally, in *Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritization of research needs,* Eerkes-Medrano, Thompson, and Aldridge state that, "Initial field and laboratory studies have demonstrated that five species of freshwater invertebrates, one species of freshwater fish, nine species of brackish fish, and one species of ampidromous fish can ingest microplastics" (2015).

Microplastics in freshwater ecosystems

Only in the last few years have scientists begun to study how microplastics are affecting freshwaters. According to Eerkes-Medrano et al., this pollutant has been detected in freshwater systems in North America, Europe, and Asia, with the first biotic studies revealing that microplastic particles are indeed being ingested by a range of freshwater fauna (2015). In their 2015 review, the authors summarize where many of the recent studies have found microplastic debris in both freshwater and its sediment:

> Microplastics have been found in: North America, in the Los Angeles basin (Moore et al., 2011), the North Shore Channel of Chicago (Hoellein et al., 2014), the St. Lawrence River (Castañeda et al., 2014) and the Great Lakes (Zbyszewski and Corcoran, 2011;

> Zbyszewski et al., 2014; Eriksen et al., 2013); in Europe, in Lake Geneva (Faure et al., 2012), the Italian Lake Guarda (Imhof et al., 2013), the Austrian Danube river (Lechner et al., 2014), the German Elbe, Mosel, Neckar, and Rhine rivers (Wagner et al., 2014), and the UK Tamar estuary (Sadri and Thompson, 2014); and in Asia, in Lake Hovsgol, Mongolia (Free et al.,2014). (Eerkes-Medrano et al. 2015, p. 65)

To date, freshwater microplastic studies have varied greatly in their magnitude and research focus, with little standardization in sampling methods and laboratory analysis. Results generally provide plastic count, concentration, and/or plastic weight; with the pieces often sorted by type (foam, line, pellet, bead, fragment, etc.), chemical composition, color, and/or size. In *Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification*, Hidalgo-Ruz et al. (2012) provide a thorough overview (using 68 research articles) of the methods, for both microplastic collection and analysis, that have been used in past studies. Although the context of the review is for marine research, the article's findings remain in large part applicable to the study of microplastic pollution in the freshwater setting.

Great Lakes Studies

A study conducted in 2012 in part by the 5 Gyres Institute (Eriksen et al. 2013) investigated the premise that microplastics are finding their way into the Laurentian Great Lakes, making their study the first open-water survey of plastic pollution in this lake system. Collecting surface water samples using a 333 lm mesh manta trawl at 21 stations over a 1300 km expedition, the authors reported an average abundance of nearly 43,000 microplastic particles/km^2. They note that the samples collected from Lake Erie were consistently the most concentrated in comparison to those taken from the other two lakes in the study, Superior and Huron. Many of these particles were multi-colored spheres believed to come from consumer

products that contain exfoliating “micro-beads” (Eriksen et al. 2013). These particles, along with coal ash collected in the samples, are suspected to originate from the urban effluent and coal burning power plants of the heavily urbanized surrounding watersheds. These watersheds of the major surrounding cities ultimately flow into the St. Lawrence River, and from there, into the North Atlantic Ocean (Eriksen et al. 2013).

Another study, published in 2014, looked at plastic pollution along the shorelines of the Great Lakes basin. Zbyszewski et al. (2014) sampled and monitored the shores of Lake Huron, Erie and St. Clair, where they concluded that a considerable amount of plastic were transported and deposited by waves during storm events. They note that the higher abundance of PE (polyethylene) and PP (polypropylene) along the Great Lakes’ shorelines could be attributed to their low density, and therefore, ability to float. This finding is similar in marine plastic debris collected, as these two polymers are the most widely produced globally (Zbyszewski et al. 2014). The authors collected 5,602 plastic particles, pertaining primarily to litter from plastic fragments and intact products, as well as some plastic pellets. Additionally, plastic fragments collected falling within the 0.5-2 cm size class were found along the shores of all three lakes, with Lake Erie containing the highest relative abundance. They attribute the occurrence of the plastics found is again due to surrounding urban waste and factory and transport spillage (Zbyszewski et al. 2014).

In 2016 a large-scale open water study that spanned the entirety of Lake Michigan was conducted, in a similar fashion to that of Eriksen et al. (2013), described above. Researchers collected 59 surface water samples using a manta trawl (333 µm mesh) towed for 30 minutes (Mason et al. 2016b). Using a modified version of the National Oceanic and Atmospheric Administration (NOAA) marine debris protocol, plastics were sorted into three different size

classes and five different particle types (fragment, pellet, fiber/line, film, foam) (Mason et al. 2016b; Masura et al. 2015). Using advanced microscopy techniques (SEM/EDS and FTIR analysis), plastics were found in all but one of the 59 samples, with plastic concentrations ranging from ~1,400 to 100,000 particles/km^2 (Mason et al. 2016b). They found an average of 17,276 particles/km^2 and a relatively even distribution of plastic particles across the lake's surface, which can likely be attributed in part to its long residence time (99 years) (Mason et al. 2016b).

Lastly, the authors state that their findings were consistent with those of Eriksen et al. (2013) in that the majority of particles collected were within the smallest size class classification (0.355–0.999 mm). However, there were notable differences in the abundance of particle types found. The Lake Michigan study found fragments to be in highest abundance, with fibers being the second most abundant, while Eriksen et al. (2013) found pellets to be the dominating particle type collected in the other Great Lakes (Mason et al. 2016b).

River Studies

According to Lechner et al., "A significant portion of the terrestrial plastic is transported to the seas by rivers. Nevertheless, quantifications of plastic loads in rivers found in primary literature are minimal" (2014, p. 177). In a recent study, Lechner et al. conducted a two-year survey of microplastics in the river Danube, Europe's second largest river (2014). Using stationary conical driftnets (500 µm mesh), they recovered a total of 17,349 plastic items from their 951 samples taken, with a mean plastic abundance of (n= 17,349; mean ± S.D: 316.8 ± 4664.6 items per 1000 m^{-3}) (Lechner et al. 2014). This presented the first evidence that large rivers transport considerable amounts of microplastics to the oceans. The researchers found that the mean plastic abundance and mass in the river were greater than that of drifting larval fish,

which were also collected. The plastics consisted largely of industrial raw material such as flakes, pellets, and spherules/beads (Lechner et al. 2014). With an estimated plastic input of 4.2 t per day, the authors stated that in the worst case scenario, the Danube could transport more plastic to the Dead Sea in one year than the entire plastic load of the North Atlantic Gyre (Law et al. 2010; Lechner et al. 2014; Wagner et al. 2014).

Sadri and Thompson (2014) sampled England's Tamar Estuary for floating plastic debris and found a total of 204 pieces of suspected plastic, 84% of which were confirmed to be plastic. Of those pieces, microplastics of multiple types made up 82%, with the 1-3 mm size category being the most abundant (Sadri & Thompson 2014). Their samples were collected from surface waters near the mouth of the River Tamar using a manta net (300µm mesh) towed for 30 minutes during the maximum flow period, sampling both spring and neap tides. Overall, the average concentration of plastic was $0.028/m^3$ (Sadri & Thompson 2014).

Morritt, Stefanoudis, Pearce, Crimmen, and Clark (2014) took a different approach on the River Thames, also in England, by looking at the amount of submerged plastic being transported by the river. While their focus was not on microplastics specifically, their study found significant amounts of plastic debris in the river using eel fyke nets anchored into the river bed. They collected 8490 submerged plastic items in total over a three month period from seven different localities. They noted that the sites in the vicinity of sewage treatment works were the most contaminated (Morritt et al. 2014). The authors conclude:

> There is little doubt that significant quantities of litter, especially plastics, are moving down the River Thames and thus providing a major input of such debris to the North Sea…but of increasing concern are the less obvious effects, including the gradual fragmentation of macroplastics generating microplastic fragments which ultimately find

their way into the bodies of a wide range of marine organisms. (Morritt et al. 2014, p.199)

In the United States, Yonkos, Friedel, Perez-Reyes, Ghosal, and Arthur (2014) also studied microplastic concentrations in the estuarine environment, looking at four estuarine rivers in the Chesapeake Bay. They hypothesized that, "microplastic concentration would be higher in proximity to urban sources, and vary temporally in response to weather phenomena such as storm events" (Yonkos et al. 2014). Using a manta net (0.33mm mesh) to collect surface samples from four sample sites with diverse watershed characteristics (e.g., land use and population densities), microplastics were collected in all but one of the 60 samples. According to the authors, concentrations ranged over three orders of magnitude (<1.0 to >560 g/km^2) and showed statistically significant positive correlations with population density and proportion of urban/suburban development within watersheds (Yonkos et al. 2014). They also note that major rain events resulted in the greatest microplastic concentrations at three of their four sites.

In 2011, Moore, Lattin, and Zellers studied the quantity and type of plastic debris occurring in the Los Angeles and San Gabriel Rivers, the two main rivers that drain the Los Angeles basin. Both wet and dry weather sampling was conducted at each site at the middle and edge of the channel, and at the surface and bottom, according to the authors. A variety of net types were utilized for the different locations, all with a mesh size less than 1 mm (Moore et al. 2011). A General Oceanics flowmeter was used to determine flow and the original sampling time was 15 minutes. Regarding their results, Moore et al. states, "The greatest abundance and density of debris occurred on November 22, 2004 in the LA River after a light rain. Particles less than 5mm in size were 16 times more abundant than those greater than 5mm, and weighed three times more than the larger particles" (2011, p. 68). The total abundance of microplastic particles

collected that day in the LA River, from all sampling devices, was 12,932 pieces/m^3 (Moore et al. 2011).

McCormick, Hoellein, Mason, Schluep, and Kelly (2014) examined the highly urbanized North Shore Channel (NSC) in Chicago, Illinois with the goal of measuring the microplastic concentration in an urban river and assessing WWTP effluent as a potential point source. The authors describe the NCS as:

> A 12-km man-made channel built in 1910 that receives water from Lake Michigan at Waukegan, IL and joins the North Branch of the Chicago River at Foster Ave in Chicago, IL. Treated wastewater effluent from the Terrance J. O'Brien Water Reclamation Plant flows into the NSC approximately 5.6 km upstream of its confluence with the Chicago River. (McCormick et al. 2014, p.11864)

Two neuston nets (333 µm mesh) were used to collect microplastics on a single day, September 13, 2013, and were deployed at the same time behind a stationary boat for 20 minutes, both upstream and downstream of WWTP effluent (McCormick et al. 2014). According to the authors, each net sample contained microplastic and the concentration downstream of the WWTP effluent was greater than upstream, with mean (± SE) microplastic concentrations being 1.94 (0.81) m^{-3} upstream and 17.93 (11.05) m^{-3} downstream (McCormick et al. 2014). Additionally, they believe that the higher abundance collected at the downstream site is an indicator that WWTP effluent was a point source of microplastic pollution to the river. These included, "fibers and pellets associated with synthetic textiles and personal care and cleaning products, respectively" (McCormick et al. 2014, p.11867). Lastly, the microplastic particles they collected at their study site were colonized by dense bacterial biofilms.

Most recently, Baldwin, Corsi, and Mason (2016), in affiliation with the U.S. Geological Survey and the State University of New York at Fredonia, conducted an expansive study on the quantity and morphology of floating micro- and macroplastics in 29 tributaries to the Great Lakes. The study covered six different states, including two sites in Indiana, with samples that represented various land covers, population densities, hydrologic conditions, and wastewater effluent contributions (Baldwin et al. 2016). Samples were collected from each tributary three or four times using a neuston net (333 μm mesh) and collected by boat, bridge, or wading. According to the authors, "Plastics were found in all 107 samples, with a maximum concentration of 32 particles/m^3 and a median of 1.9 particles/m^3. Ninety-eight percent of sampled plastic particles were less than 4.75 mm in diameter and therefore considered microplastics" (Baldwin et al. 2016, abstract). Fibers/lines were the most common plastic type collected and made up on average 71% of each sample. This was a surprising contrast to the findings of Eriksen et al. (2013), described previously, from their study of the surface waters of the Great Lakes.

Two recent reviews of the current state of the knowledge on microplastics in freshwater ecosystems have acknowledged immense knowledge gaps in the limited existing research (Eerkes-Medrano et al. 2015; Wagner et al. 2014). This is problematic since, according to Wagner et al., "A rough estimation predicts that 70% to 80% of marine litter, most of it plastics, originate from inland sources and are emitted by rivers to the oceans" (2014, p. 3). The authors believe that while inland sources of microplastic pollution have not been thoroughly investigated, WWTPs and urban, agricultural, touristic, and industrial runoff are likely major contributors (Wagner et al. 2014). Further research on limnologic ecosystems is greatly needed to better understand the sources and fate of freshwater microplastic debris (Wagner et al. 2014).

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RESEARCH METHODS

Site selection

The chosen site was selected because there are currently no existing studies that have sampled for microplastics in Indiana's White River. Additionally, the West Fork of the White River contains subwatersheds that are urban, suburban, and rural. This study was conducted at three locations within the watershed of the West Fork White River basin in Delaware and Marion Counties, Indiana (Fig. 1). Sampling took place from August to November of 2015. Sites 1 and 2 were chosen based on their relative locations upstream and downstream, respectively, of the Muncie Water Pollution Control Facility, and Site 3 was chosen for its more urban and highly

populated watershed. Feasibility and safety also played a role in choosing the specific bridges used for the sampling locations.

Site description

The West Fork White River

All three sampling locations are located along the West Fork White River, which fall within the Upper White (River) Watershed (HUC 05120201). This watershed has a drainage area of 2,724 square miles and 356 miles of flowing water. Its land use is approximately 60% agriculture and 25% developed land (Fig. 2 & 3) (Table 1). Further, the three sites fall within two subwatersheds (HUC-12). Sites 1 and 2 are located in York Prairie Creek-White River, which has 14 square miles of developed land, 53% of its total area. Site 3 is located in Pogues Run-White River, which has 23 square miles of developed land, contributing to 99% of its total area (Wikiwatershed 2017) (Fig. 4 & 5).

Indiana's White River, consisting of an East and West fork that both flow in a general southwesterly direction, is a major tributary to the Wabash River (Fig. 6). Draining 11,400 square miles, the White River flows for a combined 544 miles to its confluence with the Wabash, its two forks converging northeast of Petersburg, Indiana (IDEM Office of Water Quality 2014). Starting in a farm field in eastern Randolph County, the West Fork of the White River grows rapidly in size as it travels through a highly agricultural landscape. The city of Muncie is the first major urban area that influences the White River. Here, it is large enough to be used as a drinking water source, and due to the city's history of factory pollution from the early 20th Century, Muncie has focused major efforts on restoring its water quality (IDEM Office of Water Quality 2014). As explained in the *Indiana State Nonpoint Source Management Plan*:

> As the West Fork White River progresses on its course through Madison, Hamilton and Marion Counties, it grows larger from the contribution of major tributaries such as Killbuck Creek, Duck Creek, Pipe Creek, Fall Creek, Cicero Creek, Stony Creek, Eagle Creek, and White Lick Creek, and flows through the cities of Anderson, Noblesville, Fishers and Carmel into Indianapolis. (IDEM Office of Water Quality 2014, p. 18)

The White River serves as part of the drinking water supply to the city of Indianapolis. However, multiple urban issues, such as pathogens from combined sewer overflows (CSOs) and phosphorus from lawn fertilizers, have led to various pollution problems in the river. Despite this, recreation on the White River, especially angling, is still relatively widespread (Hoffman 2005; IDEM Office of Water Quality 2014).

Sampling locations

The first sampling site is the furthest upstream, located at the S. Nichols Avenue Bridge in Muncie, IN (40N 11' 6" 85W 24' 42") (Fig. 7). According to USGS StreamStats data, the area that contributes flow to the point sampled, the drainage area, is 4.41% urban and drains 244.27 square miles (Fig. 8). The riparian zone for this site has one wooded bank, with the other consisting of grass and a paved trail (part of Westside Park) (Fig. 9). The bridge is a two-lane road and there is a train overpass just upstream. The water here is generally shallow, consisting of riffle, run and some glide habitats, and is divided by a sand bar. The substrate appears to be a sandy silt with cobble. Site 1 is located around 1.5 miles upstream of the Muncie Water Pollution Control Facility.

The second sampling site, located at the S. Nebo Road Bridge in Yorktown, IN (40N 11' 9" 85W 27' 43"), is located around 1.1 miles downstream of the Muncie Water Pollution Control facility. The total drainage basin area at this point is 4.77% urban and drains a total of 245.94

square miles (Fig. 10) (USGS StreamStats 2017). This bridge is a four-lane road and the riparian zone consists of one residential grassy bank, with the other more heavily wooded (Fig. 11). There is a large rocky island in the middle of the river and the water is generally shallow, with silt and cobble substrate. The habitat consists of riffles, runs, and pools.

The third sampling site is located at the Oliver Avenue Bridge in Indianapolis, IN (39N 45' 30" 86W 10' 25") (Fig. 12), making it the site furthest downstream. At this sampling point, the total drainage basin area is 10.7% urban and drains 1632.73 square miles (Fig. 13) (USGS StreamStats 2017). The riparian zone consists of one wooded bank, the other containing grass and a paved trail (part of Riley Park). The bridge is a four-lane road with a dam/spillway immediately upstream. The water is significantly deeper than at the first two sites, consisting of primarily glide and run habitats (Fig. 14 & 15). Indianapolis, with an estimated population of 853,173 as of 2015, has the most densely populated watershed of the three sampling sites.

Sampling procedure

Over the course of four months, from August through November 2015, fifteen microplastic samples, plus duplicates, were collected at the three sampling sites described above. At each site, surface water samples were taken from the site's bridge over the course of 10 minutes each. All samples were collected during daylight hours and not within 48 hours of a runoff event. Samples were taken using a Wildco stationary stream drift net (39" in length, 18" wide, and 12" tall, 363µ mesh) with a detachable mesh dolphin bucket (368µm mesh) at the end. This mesh size falls within a commonly used size range in other microplastic studies (Baldwin et al. 2016; Hidalgo-Ruz et al. 2012). Modifications were made to the net in order to allow for deployment from a bridge and for flotation (Fig. 16). The net is similar to a neuston net or manta trawl, often used in larger pelagic or river studies.

A digital mechanical flowmeter (2030R, General Oceanics, Miami) was attached across the mouth of the net to measure the velocity of the water entering (Fig. 17). The total volume of water being filtered through the net was calculated using the width and height of the net, the duration of the sample, and the velocity (Baldwin et al. 2016; Lechner et al. 2014). After the first sampling event (sampling all three locations), a low-flow rotor was purchased to allow for a more accurate flow reading, due to issues with low velocity (approximately <10cm/sec) at all three sites. After 10 minutes, the net was rinsed from both the outside and inside, using a pressure sprayer filled with tap water, into the 200 ml dolphin bucket at the end of the net (Baldwin et al. 2016). The plastics and organic debris collected were rinsed from the bucket into a sealed glass jar using deionized water and a squirt bottle, and then placed on ice for transport to the laboratory (McCormick et al. 2014) (Fig. 18). This process was repeated at each site for the duplicate sample. At least three samples were not utilized due to flowmeter malfunction. A total of 15 samples and 15 duplicates were successfully collected.

A water sample was also collected at each sampling event using a standard grab sampler deployed from the bridge. This was used to assess water temperature at the time of sampling. Air temperature was noted and river discharge for each location was estimated using data provided by the USGS steam flow gages at sites 03347000 (White River at Muncie) and 03353000 (White River at Indianapolis).

Laboratory analysis

Microplastic studies have employed numerous analytical methods for separating and identifying microplastic pieces from environmental samples, due to the infancy of the field (Baldwin et al. 2016). Processing and laboratory analysis of the collected samples were completed using a method developed by the National Oceanic and Atmospheric Administration,

with minor adjustments made (Masura et al. 2015). According to Baldwin et al., a number of recent studies have also used this method (2016). The Marine & Environmental Research Institute's *Guide to Microplastic Identification* was also utilized to aid in the microscope identification process. Sorting and analysis of the samples was conducted in a laboratory to separate out the non-plastic debris, and count and categorize the plastic particles into six categories based on their morphology: fragments (broken down larger plastics), beads (micro-beads from personal care products, bead blasting, etc.), fibers (synthetic textiles), films (plastic wrapping and bags), foam (foam packaging and cups), and pellets (preproduction pellets 5 mm and larger) (Baldwin et al. 2016; Lechner et al. 2014; Zbyszewski et al. 2014).

As Baldwin et al. explains, morphology-based categorization was utilized since attributing a source to the plastics found is difficult to do using visual identification as a primary tool. Spectroscopic methods, like Fourier transform infrared (FTIR) and Raman spectroscopy, which identify particles by polymer composition, allow for additional verification and less subjectivity (Baldwin et al. 2016). However, multiple studies have chosen to use visual identification, despite its potential to both over and under-estimate certain particle types, as it is less time consuming and less expensive (Baldwin et al. 2016; Eriksen et al. 2013; Mason et al. 2016b).

Wet Sieving

A combination of four primary steps were used to conduct this analysis. First, each sample was divided into two size categories by filtering the contents of each sample jar through stacked stainless steel sieves (mesh sizes of 500 µm and 250 µm) (Fig. 19). These sizes were chosen to highlight the smaller spectrum of microplastic pollution (250-500 µm) in size, and also fall within what Hidalgo-Ruz et al. (2012) explains as a commonly used size range. An

additional larger sieve was not utilized in order to minimize sample processing time and avoid potential loss of microplastics that could remain on the sieve. It should be noted that all pieces counted in this study were smaller than 5 mm in size (in diameter for fibers), the maximum range for microplastic pollution.

The sieves were rinsed thoroughly using a squirt bottle filled with distilled water. Material retained on each sieve was scraped off using a metal spatula and spoon, and rinsed (using a minimal amount of water) into individual labeled and weighed 500-ml beakers. The beakers were then covered with aluminum foil and placed in a drying oven set at 90°C, for approximately 24 hours, until dry (Fig. 20). The mass of the dried solids was determined by subtracting the mass of the tared beaker from the mass of the beaker containing the dried solids (Masura et al. 2015).

Wet Peroxide Oxidation (WPO)

The second step involved the use of wet peroxide oxidation to degrade any organic material collected in the samples (Fig. 21). For both size categories, 20 ml of aqueous 0.05 M Fe(II) solution and 20 ml of 30% hydrogen peroxide was added to the beaker containing the dried sample. After standing on the lab bench at room temperature for approximately five minutes, a stir bar was added and the mixture was heated to 75º C on a hotplate. When gas bubbles were observed at the surface, the beaker was removed from the heat until boiling subsided. Distilled water was added with a squirt bottle if the reaction began to overflow the beaker. The mixture was again heated to 75º C for an additional 30 minutes. Next, approximately 6g of salt (NaCl) per 20 ml of sample (~12 g) was added to increase the density of the solution. The mixture was heated once more to 75º C until the salt was dissolved (Masura et al. 2015).

Density Separation

The third step utilized was the process of density separation. Three density separators were created using glass funnels (122 mm in diameter) fitted with a 50 mm segment of latex tubing at the bottom of the stem (Fig. 22). A pinch clamp was attached to control the flow of liquid from the funnel (Masura et al. 2015). The WPO solution from the previous step was then transferred to a density separator, rinsing the beaker thoroughly with distilled water to ensure all remaining solids were transferred. The density separator was covered with aluminum foil to prevent contamination and the solids were allowed to settle overnight. The settled solids, presumably too heavy to be plastics, were then drained and discarded. The floating solids and plastics were drained into a clean custom-made sieve, either 250 µm or 500 µm mesh size, depending on the sample's size category (Fig. 23). Sieves were made using cut sections of PVC pipe, approximately 75 mm in diameter and 25 mm in length, and nylon mesh of the appropriate size, attached at one end using a gel-type superglue (Masura et al. 2015) (Fig. 24). Density separators were rinsed thoroughly with distilled water to transfer all solids to the appropriate-sized sieve. The sieve was then loosely covered and allowed to dry for 24 or more hours, until the contents were dry. It should be noted that one portion of a sized-divided sample was spilled during processing.

Microscope Examination

Visual sorting of the samples was conducted with the use of a stereoscope (dissecting microscope) at 40X magnification or higher (Hidalgo-Ruz et al. 2012; Masura et al. 2015; McCormick et al. 2014). Tweezers were used to remove all identifiable plastics from the sieves

for both size categories and transfer them to labeled glass vials for storage. Each sample was examined for approximately 30-45 minutes under the microscope (Fig. 25).

The identification process was aided by the use of guidelines published by the Marine & Environmental Research Institute (MERI). Microplastic characteristics were defined as: "1. Small size (largest dimension ≤5 mm), 2. No cellular or organic structures visible, 3. Fibers should be equally thick throughout their entire length, 4. Particles should exhibit clear and homogenous color throughout" (Hidalgo-Ruz et al. 2012; Marine & Environmental Research Institute n.d., p. 3). The nylon sieve mesh was used as a grid and examined from left to right, from top to bottom, to ensure pieces were not missed or double counted (Marine & Environmental Research Institute n.d.).

According to the MERI guidelines, plastic pieces are relatively flexible and will not break when prodded. Therefore, particles that broke when touched with tweezers were not counted as plastic. Any detritus and salt piles that existed on a sieve were carefully picked through as best as possible to avoid missing any plastics. Additionally, the "hot needle test" (based on De Witte et al. 2014) was heavily relied upon in distinguishing between plastic and non-plastic particles, especially between natural and synthetic fibers (Fig. 26). The guidelines state, "In the presence of a very hot needle, plastic pieces will melt or curl. Biological and other non-plastic materials will not" (Marine & Environmental Research Institute n.d., p. 5). The type (from one of the six categories mentioned previously) and color of each piece collected was recorded, and the total plastic count for each sample was calculated. Recording plastic weight for each sample was not feasible due to the loss of pieces through the sieve during microscope identification and from the use of the hot needle.

Quality assurance and control

When studying microplastics, it is necessary to be aware of the potential for sample contamination from a number of sources. Precautions were taken during this study to avoid contamination from within the lab. Samples were processed under a fume hood and always remained covered when not in use. Other equipment and tools used in the lab were also covered or washed before use. A cotton lab coat was worn at all times during sample processing.

While not utilized in this study, Baldwin et al. collected both field and lab blanks to assess the potential for cross-contamination between samples from the nets used, and also for potential contamination from laboratory containers or the air itself. They found an average of 17 plastic particles from the five field blank samples and determined that cross-contamination from sample to sample was relatively low, when compared to the average number of plastic particles in most of their environmental samples (368). Additionally, the eleven laboratory blanks that were analyzed alongside the environmental samples revealed no plastic particles, indicating the laboratory itself to be a low risk of contamination (Baldwin et al. 2016). The methods used in Baldwin et al. are similar to those used in this study, and it could therefore be assumed that the risk of contamination was also low.

Statistical data analysis

For this study, as with numerous other microplastic studies, plastic concentrations were reported in particles, or items, per cubic meter (item/m^3) (Baldwin et al. 2016; Hidalgo-Ruz et al. 2012). The particles collected in each sample were averaged with those collected in the sample's duplicate, resulting in 15 sampling events analyzed. Data analysis was conducted using SPSS software (IBM Corp., Armonk, NY) with statistical significance reported at $p = 0.05$. Because of

the limited sample size (N=15), a Kruskal-Wallis test by ranks (equivalent to a non-parametric one-way ANOVA test) was utilized to evaluate the spatial variation between the concentrations found at the three sampling sites, using site location and plastic concentration as variables (Baldwin et al. 2016). This test was also used to look for a significant relationship between plastic concentration and the month a sample was collected. All analyses were completed for the smallest pieces, those between 250-500 µm in size, and again for the total pieces collected in each sample (from both the 250 and 500 µm sieves combined).

Additionally, descriptive statistics were used to find the sum of plastics by type (fragment, bead, fiber, film, foam, and pellet) for each site, as well as each type's average, its range, and its percent of the total plastics collected for that site. Lastly, the overall total for each plastic type was calculated, in addition to the microplastic totals for the 250-500 µm size category and for all of the microplastics collected as a whole.

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RESULTS

Summary of findings

Total Pieces and Types Collected

As anticipated, microplastics of numerous types, colors, and sizes were collected from all three sites sampled. A total of 145.5 plastic pieces were collected from the White River over the duration of this study, from all 15 samples. Of those pieces, 39.5 (only 27 percent), were in the 250-500-µm size range. The remainder were collected from the 500 µm sieve and were considered to be that size or larger, with the few pieces larger than 5 mm removed. As mentioned above, plastic counts, averages, and ranges by type were also calculated for each site (Table 2 & 3).

Contrary to expectation at the start of this study, synthetic fibers were found to be the overwhelmingly predominant plastic particle type collected, for both the 250-500 µm size category and in total, ranging from 64-86% of the total plastics collected at each site (Fig. 27). The 145.5 microplastic pieces collected in this study were comprised of 16 fragments, 1 bead, 111.5 fibers, 3 films, 13 foamed plastics, and 1 pellet (Figures 28-33). Some error in particle type identification is assumed due to the inherent subjectivity of visual identification. Additionally, the difficult nature of distinguishing between certain particle types (such as fragments and films) and between plastic and non-plastic particles of close resemblance (such as organic matter and natural fibers) also increases the potential for microplastic identification error. Lastly, plastic color, while not a primary focus of the study, was also noted for each sample. Translucent, white, black, and red appeared to be the most prevalent plastic colors collected on average. Again, color determination is also prone to subjectivity.

Average Concentrations

As is standard in many microplastic studies, average concentrations (items/m^3) were calculated for each site and for the study as a whole, in order to standardize the findings for comparison to other studies (Table 4). Site 2 (Yorktown) had the lowest average concentration for the smaller microplastic size range (250-500 µm), with 0.15 items/m^3, and the lowest average *total plastic* concentration, with 0.44 items/m^3. Site 1 (Muncie), with 0.24 items/m^3, and Site 3 (Indianapolis), with 0.23 items/m^3, resulted in very similar concentrations for the smaller microplastic size class. However, for the total microplastic concentration, Site 1 had an average of 0.75 items/m^3, compared to an average total microplastic concentration of 0.93 items/m^3 in Indianapolis, the highest of the three sites. The overall *total plastic* concentration for the White River, based on the three sites sampled, was 0.71 items/m^3 (Fig. 35).

Concentration Differences Between Sites

The Kruskal-Wallis test (using $\alpha = 0.05$) was used to compare the relationship between a sampling site and its plastic concentrations. For both the 250-500 µm size category ($p = 0.961$) and for the total plastics category ($p = 0.395$), there was no significant difference shown in the mean ranks given to each site. Therefore, we would *not* reject the null hypothesis, which states that there is no significant difference in plastic concentration between site locations. Plastic concentration did not differ significantly between sites for the smaller microplastics or for all of the microplastics combined.

A Kruskal-Wallis test (using $\alpha = 0.05$) also revealed that there was no significant difference shown in the mean ranks given to each sampling month for either the 250-500 µm

plastics ($p = 0.849$) or for total plastics ($p = 0.753$). Therefore, sample concentrations did not differ significantly based on the month they were collected.

DISCUSSION

Site comparison in relation to watershed attributes

Although plastic concentrations in this study did not differ significantly by sampling site, the quantities and types of plastics found are relatively consistent with those of other recent fluvial plastic studies (Baldwin et al. 2016) and contribute to the overall understanding of microplastic abundance and behavior in this unique environment. Additionally, the results aid in the understanding of how watershed attributes, such as urban development, affect microplastic pollution. Although not statistically significant, the total pieces of plastic collected and the average plastic concentration at the Indianapolis site were the highest of all three sampling locations (63 pieces and 0.93 items/m^3), as could be expected based on its larger population and largely urban subwatershed.

Baldwin et al. further supports this finding by explaining that plastic litter is more prevalent and mobile in urban watersheds due to the high amount of impervious surfaces and storm sewers that transport plastics to receiving water bodies during runoff-events. Additionally, they explain that, "Previous research (Yonkos et al. 2014) has also supported a correlation between microplastic concentrations (predominantly fragments and films) and urban-related attributes (population density and urban/suburban development)" (Baldwin et al. 2016, p. E).

Additionally, Yonkos et al. found that plastic concentrations demonstrated statistically significant positive correlations with population density and proportion of urban/suburban development with the watersheds of four estuarine rivers in the Chesapeake Bay (2014).

Effects of the Muncie Water Pollution Control Facility

Another point of interest in this study was in determining whether samples taken at Site 2 (Yorktown), which is just downstream of the Muncie Water Pollution Control Facility, would have higher microplastic concentrations than those taken at Site 1 (Muncie), which is just upstream. Since plastic concentrations were not found to differ significantly between any of the sites, there was no evidence that this facility was discharging significant amounts of microplastic pollution, resulting in significantly higher plastic concentrations at Site 2 versus Site 1. Pieces collected at both sites were nearly equal, with the Muncie site actually resulting in the higher average plastic concentration (0.44 vs. 0.75 items/m^3).

While understanding the direct sources of microplastics found in environmental studies is difficult, recent WWTP studies do verify that these facilities are a significant source of primary microplastics into the environment, despite relatively effective treatment processes. However, the lack of (micro) beads found in this study (only 1 total) is consistent with the findings of Murphy et al. 2016. Only in their grease and grit samples (which are skimmed off during primary treatment) did the authors find any microbeads. They explain that because most microbeads found in face washes contain positively-buoyant polyethylene, they will tend to float on the surface layer of wastewater, where they are easy to skim off during the grease removal stage. Additionally, they emphasize that since microbeads were not seen in the final effluent, it is likely that microbeads from personal care products may not actually have a major impact on the receiving environment if treatment processes are utilized appropriately (Murphy et al. 2016).

The authors also touch on the fact that not all wastewater goes through the treatment process at a WWTP, in the case of heavy precipitation. Like many Midwestern states, Indiana

has numerous combined sewer overflow outfalls along its waterways, like the White River, where untreated combined waste and stormwater is discharged when the volume of influent at a facility exceeds the treatable volume. Murphy et al. explains how this untreated wastewater has potential to greatly affect the amount of microplastics entering the environment. However, no studies have yet investigated stormwater overflow as it relates to microplastic pollution (2016).

Mason et al. (2016a), in their recent broad study of wastewater effluent from 17 different U.S. wastewater treatment facilities, also did not find microbeads to be prevalent in their 90 samples. They found fibers (59%) and fragments (33%) to be the most common microplastic types in their study as a whole, consistent with the study at hand. Further, they found smaller microplastics (0.125-0.355 mm) to be slightly more prevalent than larger particles (>0.355), which differed from the results of this study.

Lastly, despite the lack of WWTP-specific findings in this study, the notion that these facilities can act as a point source of microplastics to bodies of water has been demonstrated by the findings of McCormick et al. in Chicago's North Shore Channel. After sampling the channel both upstream and downstream of a WWTP, they found a high abundance of microplastics downstream of the facility, in comparison with the upstream site, indicating the effluent as a source (2014).

Comparison to other freshwater studies

This study aimed in part to capture microplastics on the smaller end of their size range (250-500 μm), which are often underestimated. A sieve to exclude plastics greater than the upper microplastic size limit (5 mm) was not utilized in order to increase processing time and avoid overall plastic loss by the use of an additional sieve. Baldwin et al. describes how past studies, in numerous aquatic environments, have shown an inverse relationship between particle size and

plastic concentration (2016). This potentially means that studies using nets with larger mesh sizes have underestimated actual microplastic concentrations. A net with a smaller mesh size than what was used in this study could have possibly resulted in a greater quantity of microplastics in the smaller 250-500 µm range.

Concentrations

The plastic concentrations that were measured from three sites on the White River (0.44-0.93 items/m^3, mean 0.71 items/m^3) are slightly lower, but comparable to those found in the few other existing microplastic river studies. Most recently, in the Great Lakes tributaries, a higher mean concentration of 4.2 items/m^3 was found (Baldwin et al. 2016). Additionally, Baldwin et al. summarizes other notable river studies:

> In Chicago's highly urbanized North Shore Channel, the mean concentrations were 1.9 and 17.9 p/m^3 *(pieces/m^3)* upstream and downstream of a WWTP, respectively. Concentrations in the Seine River upstream and downstream of Paris were 0.28-0.47 p/m^3. A mean concentration of 0.32 p/m^3 was reported for the Danube, but that study used a larger mesh size (500 µm) and did not include fibers. (2016, p. F)

Plastic Particle Types

The lack of beads found in the White River, along with the prevalence of fibers (77% of the average particles collected) is relatively consistent with the results of other fluvial studies. Fragments were the second most dominant type found (11% of the average particles collected). These findings fall in line especially with those of the aforementioned recent study on the Great Lakes tributaries. Baldwin et al. observed a similar dominance of fibers, being 71% of particles on average, and then fragments, at 17% on average (2016). Further, in Chicago's North Shore

Channel, a highly urbanized river, fibers were the most abundant plastic type collected, with fragments coming in second (McCormick et al. 2014). Also in agreement with these findings are those of Gasperi et al. (2014), where 90% of the microplastics observed in the Seine River were also fibers.

Contrastingly in the Danube, industrial plastic pellets were the dominating type of particle found, with dozens of plastic production sites throughout the multi-nation watershed, some adjacent to the river itself. Fibers, however, were not accounted for in the study (Lechner et al. 2013). The European Rhine also differed from the American studies described above, with a prevalence of plastic fragments, and plastic beads or pellets in downstream reaches, likely from plastic manufacturing, similar to those found in the Danube (Mani et al. 2015).

The difference in particle types found in other freshwater, non-fluvial studies, namely the Great Lakes, is quite substantial. Baldwin et al. explains:

> Unlike in tributary samples, fibers/lines were rare in Great Lakes samples, making up only 2% of the plastic particles on average. In a large, remote lake in Mongolia, fibers/lines made up 20% of the particles on average, which is higher than in the Great Lakes but still considerably lower than in the Great Lakes tributaries. Pellets/beads, which made up a large portion of the plastic particles in some of the Great Lakes samples (especially those from Lake Erie), were rare in tributary samples. (2016, p. F)

The authors attribute this phenomenon in part to analytical methods, but also to the actual physical properties of different plastic types and the varying hydraulics of different water bodies (Baldwin et al. 2016). They note that laboratory methods at the lab that conducted all of the analyses of the Great Lakes samples modified their analytical method after 2013, utilizing the

WPO (wet peroxide oxidation) method to isolate plastic particles instead of the previous salt water flotation method. The WPO method, used to analyze the Lake Michigan and the tributary samples, is thought to be more effective in accounting for denser particles. Despite this, the authors state that the average relative abundance of fibers in the tributary samples was still substantially greater (Baldwin et al. 2016).

The study goes on to explain that the difference in particle abundance within fluvial and lacustrine environments may be more accurately attributed to the hydraulics of river systems versus those of the Great Lakes. They explain:

> Negatively buoyant fibers made of polymers such as polyester, rayon, nylon, and cellulose acetate may remain in suspension in the turbulent flow of a river (allowing them to be captured by surface sampling) but likely settle out upon reaching the more quiescent lakes. In contrast, many foams, films, and pellets/beads are made of positively buoyant polymers such as polystyrene, polyethylene, and polypropylene, which likely remain afloat in the lakes for some time, until biofouling or adsorption of minerals increases their density and causes them to sink. It would be expected, then, that surface samples from the Great Lakes would have lower abundances of fibers relative to samples from the tributaries. (Baldwin et al. 2016, p. F-G; Ballent et al. 2016)

This explanation seems to suggest the need for further research into microplastic abundance within lakebed sediments and possible effects on the organisms that live in that habitat.

Limitations and Recommendations for Future Studies

In order to improve upon the consistency and comparability of microplastic studies of varying scales, continuing to improve upon the standardization of the both the sampling and

laboratory analysis methods will be necessary, as is mentioned widely throughout the literature. The analytical methods created by NOAA (Masura et al. 2015) and described previously provide effective and relatively non-complex guidance for researchers at any level to successfully analyze the findings of microplastic samples with ease, from both marine and freshwater studies.

While the visual inspection of microplastic samples, using the aid of a dissecting microscope, is a common method used in the literature, this step leaves the most room for human error and subjectivity. Plastic color and shape/type can be quite difficult to decipher without prior training in what to look for. Synthetic fibers especially can be easily mistaken for other items found in water samples.

Song et al. compared the use of a stereomicroscope (dissecting microscope) with the use of Fourier transform infrared spectroscope (FTIR), another method used in various microplastic studies that is known to be more difficult and expensive. Their study found that after looking at the same samples using the two different methods, the abundance of fiber was significantly ($p<0.05$) lower with FTIR than with the microscope. However, the *total* abundance of microplastics was higher by FTIR than by microscope, but not significantly so (Song et al. 2015).

The following are recommendations for future studies based on the challenges encountered in the study at hand:

- The majority of sampling issues in this study arose due to challenges with flowmeter use in low-flow situations. If feasible, a digital flowmeter would be optimal over a mechanical one.

- The “hot needle test” was effective in identifying plastics, especially in determining whether fibers are synthetic or natural. However, when using this technique it is hard to avoid melting holes in the nylon sieves or melting an entire piece of plastic that is being tested. Taking pictures of suspected plastics before performing the hot needle test, while the piece is still intact, is recommended. Also, using filter paper instead of a nylon mesh sieve for visual sorting, as suggested by the Marine & Environmental Research Institute, could be more successful.
- Both algae strands and natural fibers, like cotton, were quite difficult to differentiate from synthetic fibers without the use of the “hot needle test.”
- Quantifying microplastics by the number of pieces collected in a given volume of water, resulting in a concentration, appears more feasible than calculating the weight of the microplastics collected. Pieces are easily lost through sieve holes during microscope examination, melted using the “hot needle test,” or prove difficult to grasp with tweezers for placement into a vial for weighing.

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Conclusion

While the scale of this study is small in comparison to other microplastic studies, the results demonstrate that Indiana's White River does indeed contain comparable quantities and types of microplastic particles to those found in other fluvial studies. Namely, it further verifies the conclusion of Baldwin et al. (2016) that synthetic fibers prove to be a concerning pollution source worthy of further research to gain better insight into their environmental effects. The widespread use and laundering of synthetic clothing and the land application of treated sewage sludge, in addition to atmospheric deposition on the surrounding landscape, are all likely sources of the synthetic fibers found in the White River and other systems like it. Once there, these and other microplastic particles can be eaten by aquatic life who mistake them for food, or, when sufficiently biofouled, eventually make their way to the sediment. Here, they become available to benthic feeders or can remain in the environment for years to come. Future studies on freshwater organisms and sediments will help researchers to more clearly understand the fate and effects of the microplastic pollution that we undoubtedly know exists throughout these environments.

Table 1. Watershed Characteristics

Site	Population	Sampling Point Drainage Area (mi^2)	Drainage % Urban Development
Muncie	70,087	244.3	4.4
Yorktown	11,231	245.9	4.8
Indianapolis	853,173	1632.7	10.7

(US Census Bureau Data 2015; USGS StreamStats 2017)

Table 2. Microplastic Count by Type (250-500 µm size class)

Site	Fragments	Spherules	Fibers	Film	Foamed plastic	Pellets	Total Collected
Muncie							**11.5**
Sample average	0.3 ± 0.4	0	2 ± 0.8	0	0	0	
Range	(0-1)		(1-3)				
Total collected	1.5	0	10	0	0	0	
% of overall site total	13.0	0.0	87.0	0.0	0.0	0.0	
Yorktown							**13**
Sample average	0.1 ± 0.2	0	2.5 ± 1.2	0	0	0	
Range	(0-0.5)		(1-3.5)				
Total collected	0.5	0	12.5	0	0	0	
% of overall site total	3.8	0.0	96.2	0.0	0.0	0.0	
Indianapolis							**15**
Sample average	0.6 ± 0.7	0	2 ± 1.6	0.1 ± 0.2	0.3 ± 0.7	0	
Range	(0-1.5)		(0-4)	(0-0.5)	(0-1.5)		
Total collected	3	0	10	0.5	1.5	0	
% of overall site total	20.0	0.0	66.7	3.3	10.0	0.0	
Total	**5**	**0**	**32.5**	**0.5**	**1.5**	**0**	**39.5**

Table 3. Total Microplastic Count by Type (250-500+ µm size class)

Site	Fragments	Spherules	Fibers	Film	Foamed plastic	Pellets	Total Collected
Muncie							**42.5**
Sample average	0.4 ± 0.4	0.1 ± 0.2	7.3 ± 2.8	0.3 ± 0.3	0.4 ± 0.4	0	
Range	(0-1)	(0-0.5)	(5-11)	(0-0.5)	(0-1)		
Total collected	2	0.5	36.5	1.5	2	0	
% of overall site total	4.7	1.2	85.9	3.5	4.7	0.0	
Yorktown							**40**
Sample average	0.8 ± 1.0	0	6.9 ± 2.0	0.1 ± 0.2	0.2 ± 0.4	0	
Range	(0-2.5)		(5-9.5)	(0-0.5)	(0-1)		
Total collected	4	0	34.5	0.5	1	0	
% of overall site total	10.0	0.0	86.3	1.3	2.5	0.0	
Indianapolis							**63**
Sample average	2 ± 1.2	0.1 ± 0.2	8.1 ± 6.8	0.2 ± 0.4	2 ± 3.1	0.2 ± 0.4	
Range	(0.5-3.5)	(0-0.5)	(2.5-18.5)	(0-1)	(0-7.5)	(0-1)	
Total collected	10	0.5	40.5	1	10	1	
% of overall site total	15.9	0.8	64.3	1.6	15.9	1.6	
Total	**16**	**1**	**111.5**	**3**	**13**	**1**	**145.5**

Table 4. Average Microplastic Concentration by Site (items/m^3)

Site	(250-500 µm)	Total Plastics (250-500+ µm)
Muncie	0.24	0.75
Yorktown	0.15	0.44
Indianapolis	0.23	0.93
All Site Average	**0.21**	**0.71**

FIGURE LEGENDS

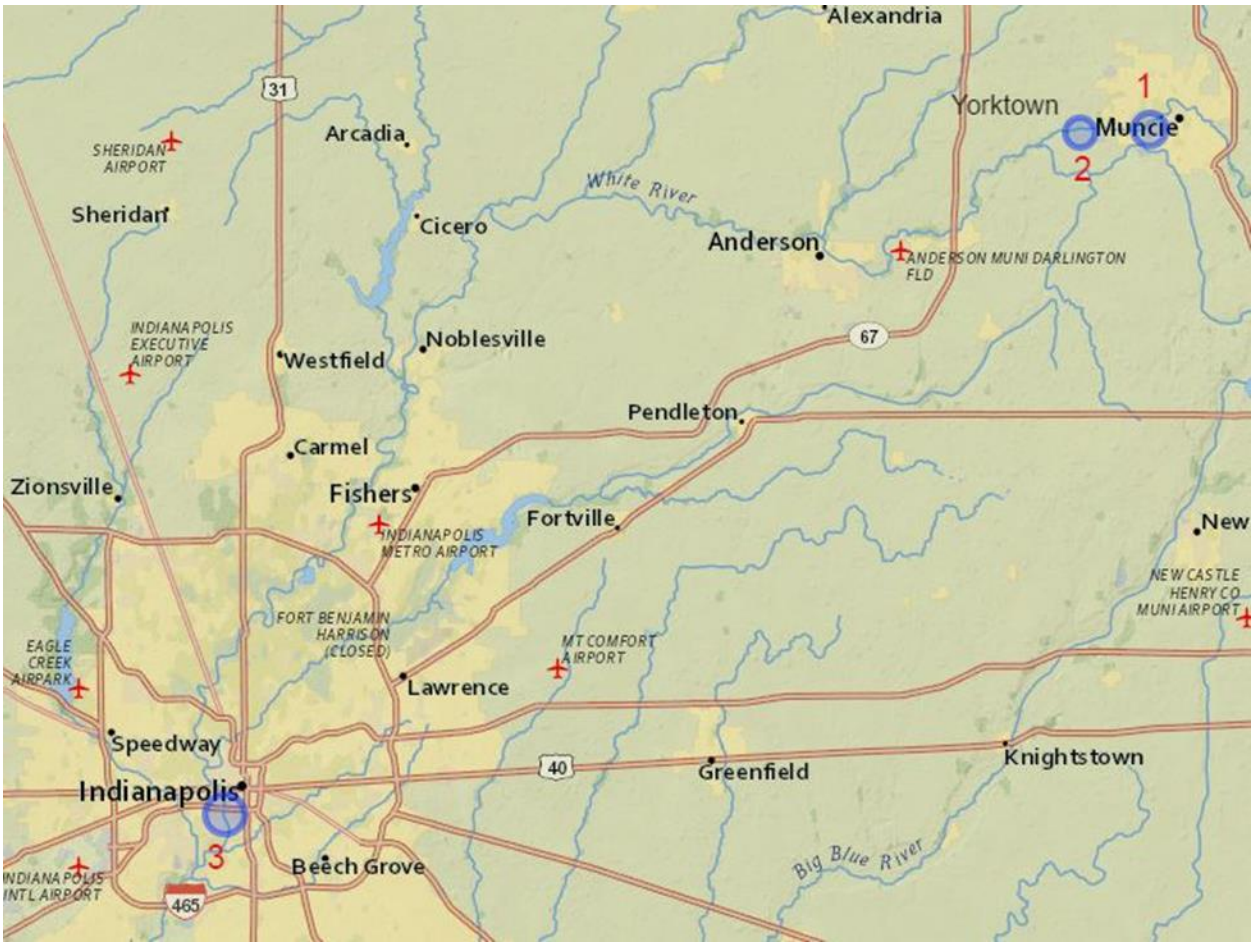
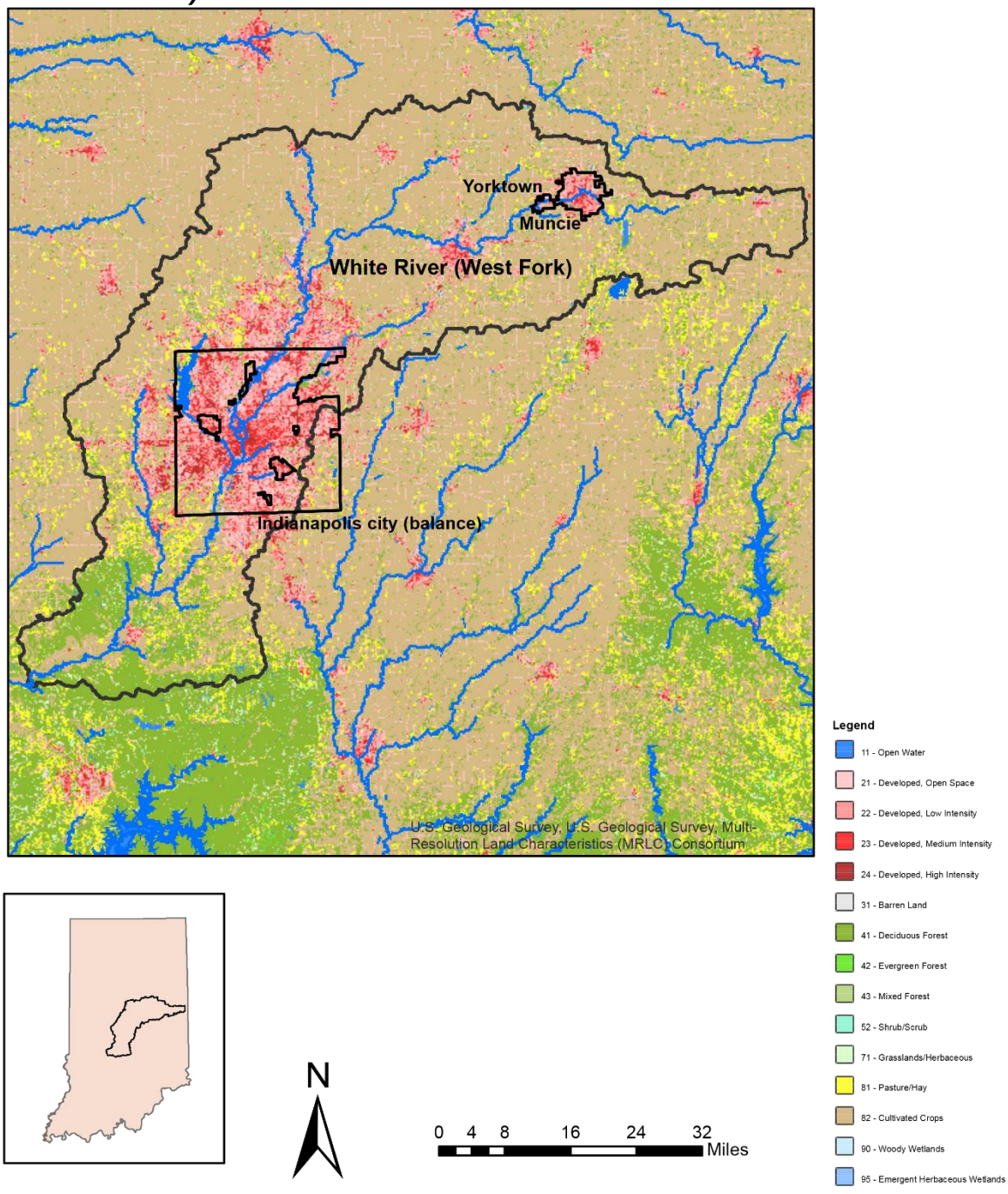

Alexandria
31
Yorktown
1
Muncie
2
SHERIDAN AIRPORT
Arcadia
White River
Sheridan
Cicero
Anderson
ANDERSON MUNI DARLINGTON FLD
INDIANAPOLIS EXECUTIVE AIRPORT
Westfield
Noblesville
67
Pendleton
Carmel
Zionsville
Fishers
INDIANAPOLIS METRO AIRPORT
Fortville
New
NEW CASTLE HENRY CO. MUNI AIRPORT
FORT BENJAMIN HARRISON (CLOSED)
EAGLE CREEK AIRPARK
MT COMFORT AIRPORT
Lawrence
Speedway
40
Greenfield
Knightstown
Indianapolis
3
Beech Grove
Big Blue River
INDIANAPOLIS INTL AIRPORT
465

Figure 1

Land Use in the Upper White River Watershed (HUC 05120201)



Mapped by: Lindsay Hylton

Figure 2

HUC-8 Subbasin: Upper White

Total Area 7,056 km^2

Land cover distribution from National Land Cover Database (NLCD 2011)

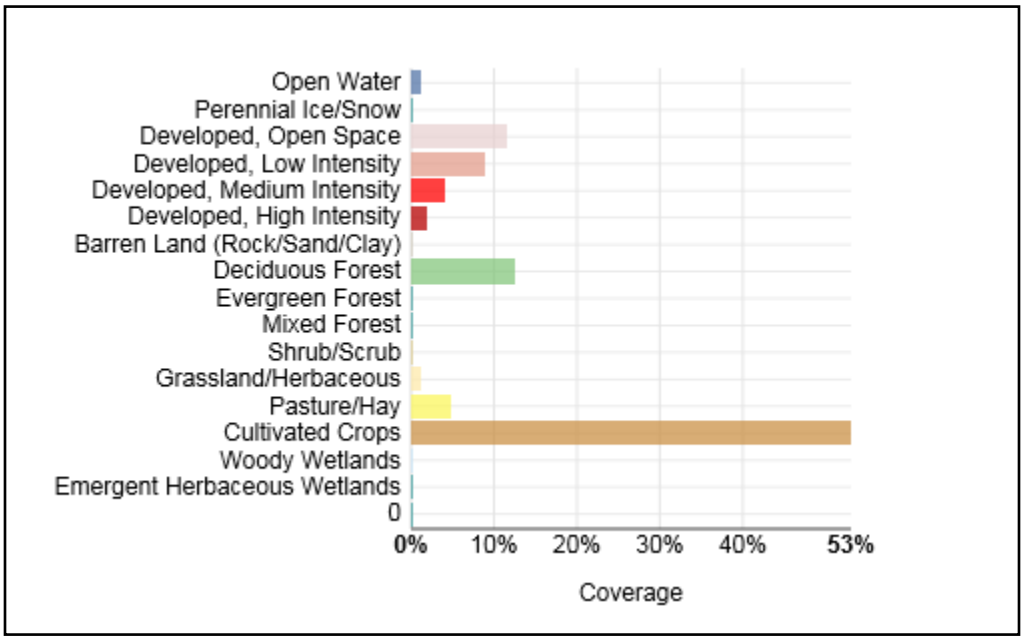


Figure 3. Adapted from Wikiwatershed.org (2017).

HUC-12 Subwatershed: York Prairie Creek-White River

Total Area 67 km^2

Land cover distribution from National Land Cover Database (NLCD 2011)

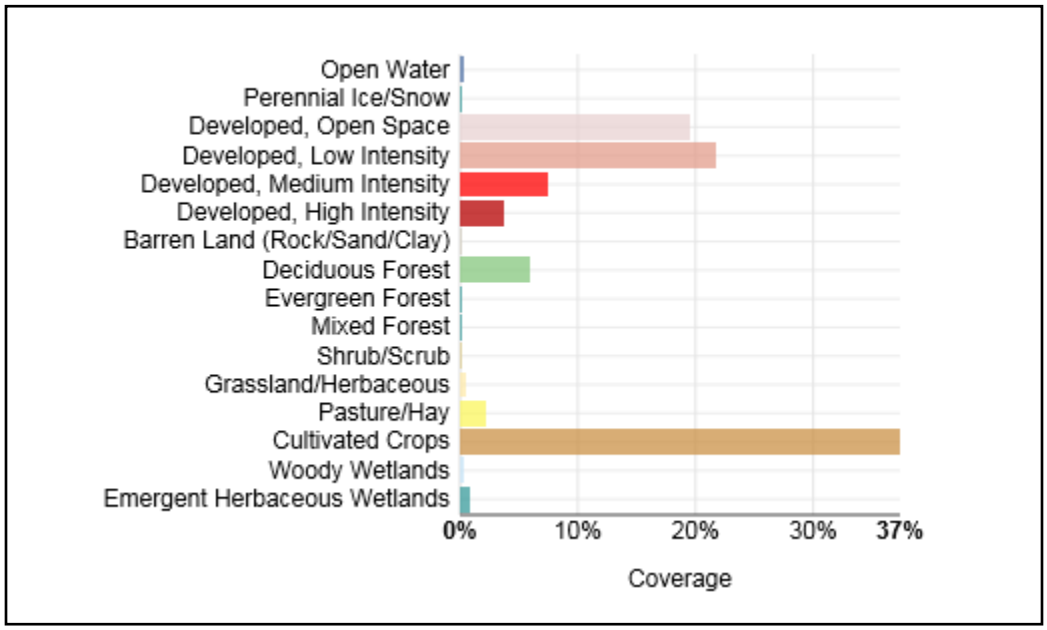


Figure 4. Adapted from Wikiwatershed.org (2017).

HUC-12 Subwatershed: Pogues Run-White River

Total Area 60 km^2

Land cover distribution from National Land Cover Database (NLCD 2011)

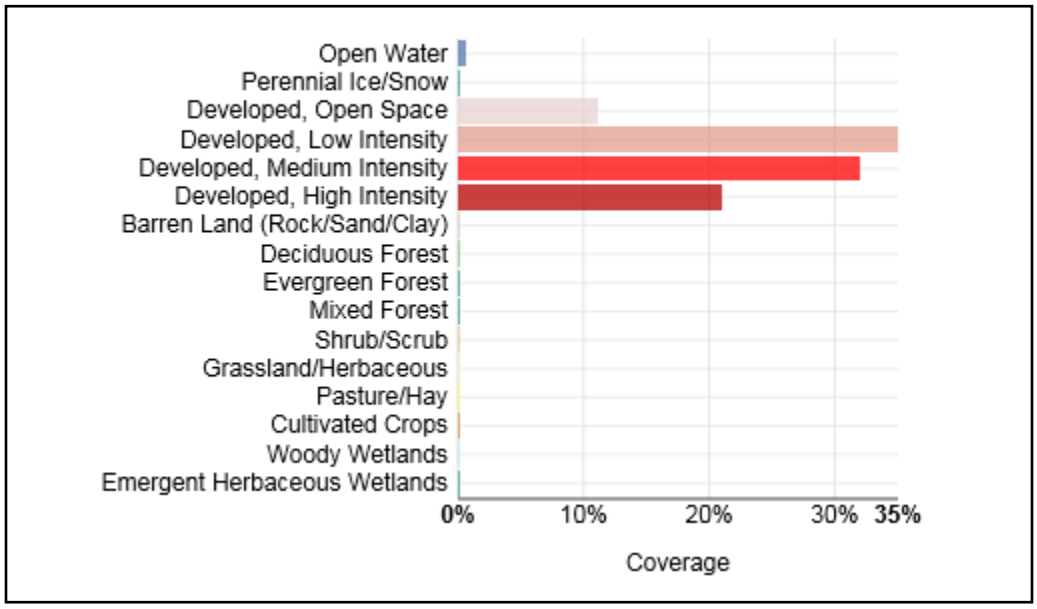


Figure 5. Adapted from Wikiwatershed.org (2017).

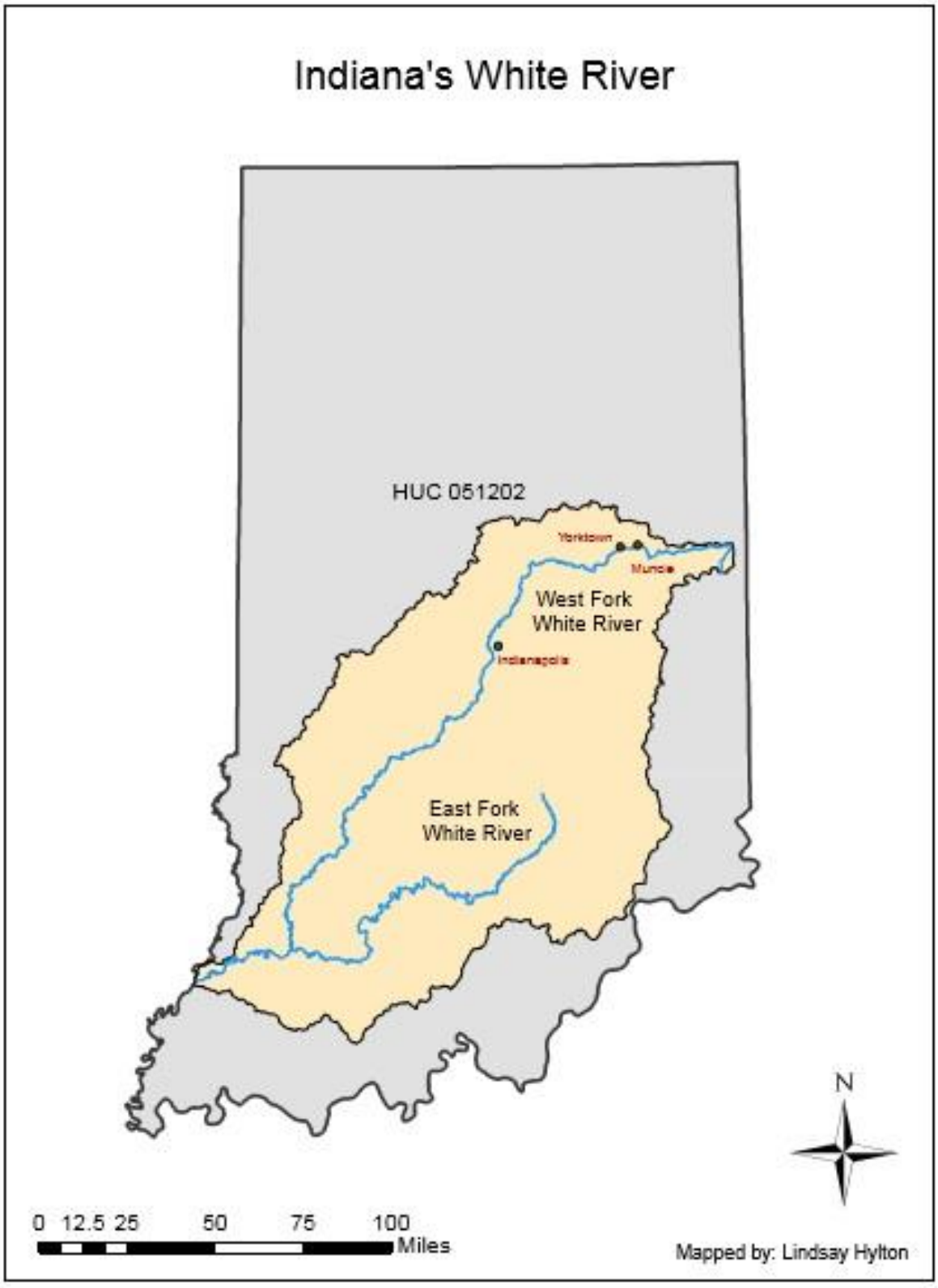

Indiana's White River
HUC 051202
Yorktown
Muncie
West Fork
White River
Indianapolis
East Fork
White River
N
0 12.5 25 50 75 100
Miles
Mapped by: Lindsay Hylton

Figure 6

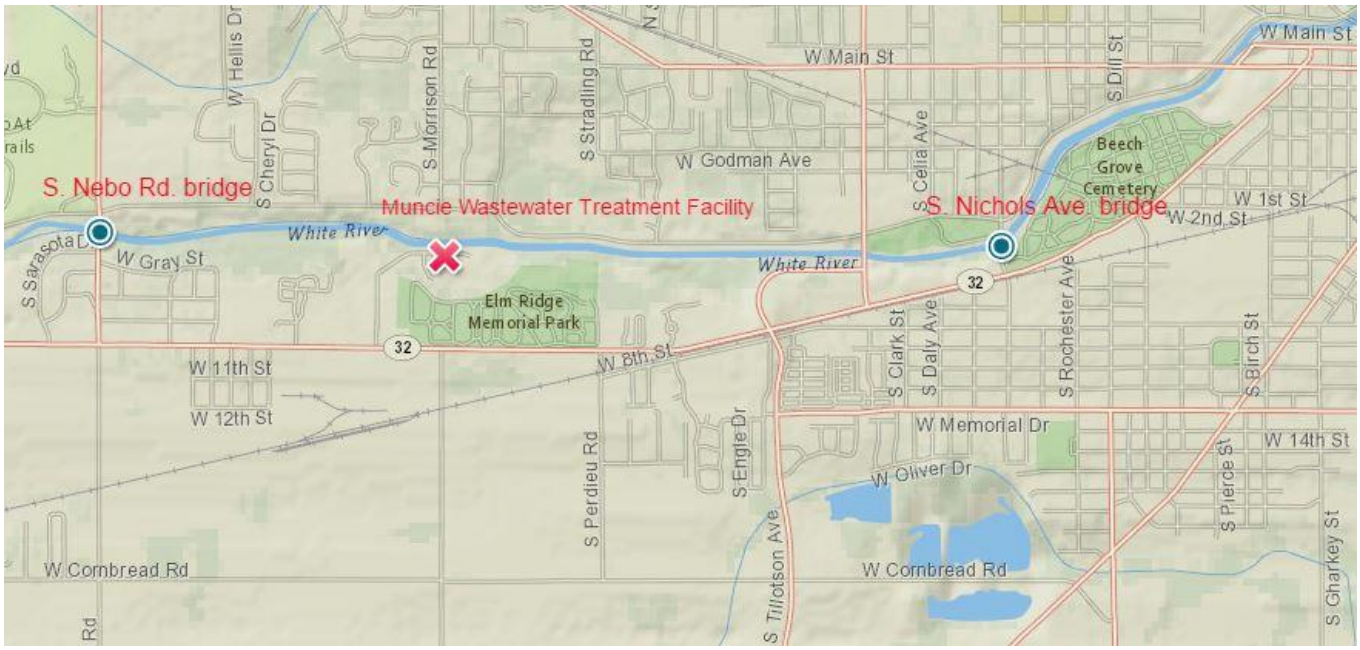


Figure 7. Adapted from Google Maps (2017).

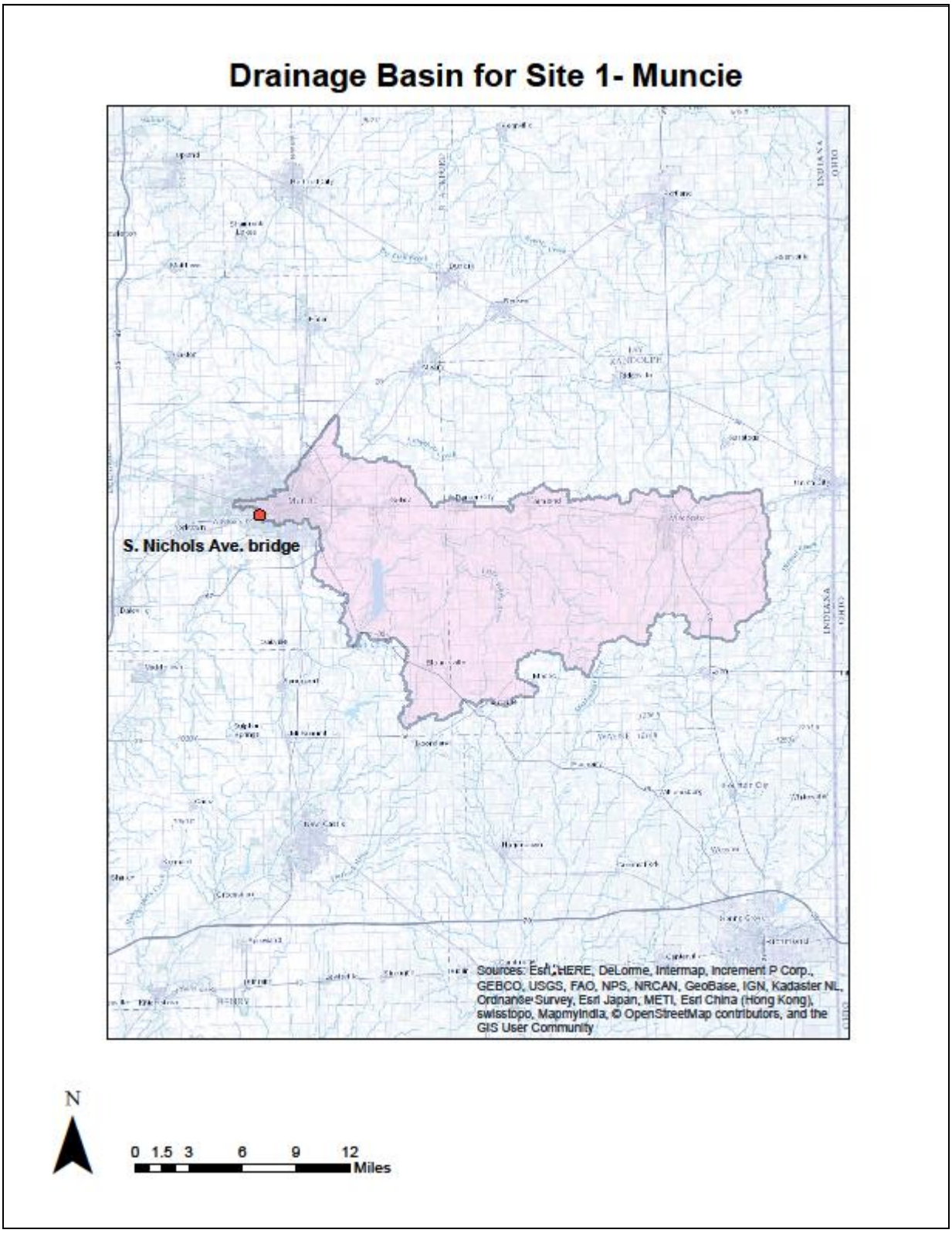

Drainage Basin for Site 1- Muncie
S. Nichols Ave. bridge
Sources: Esri, HERE, DeLorme, Intermap, Increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community
N
0 1.5 3 6 9 12
Miles

Figure 8

Figure 9

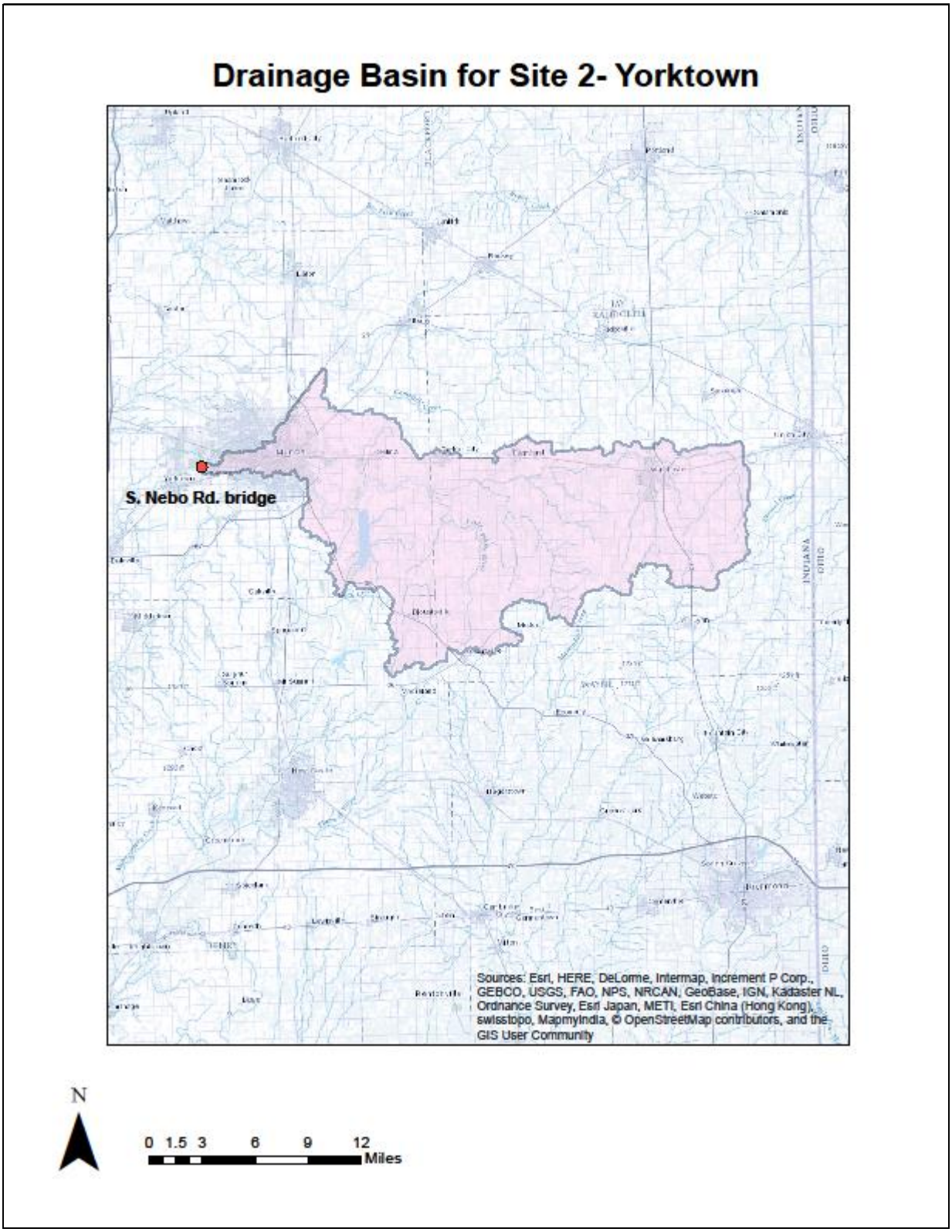

Drainage Basin for Site 2- Yorktown
S. Nebo Rd. bridge
Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community
N
0 1.5 3 6 9 12
Miles

Figure 10

Figure 11

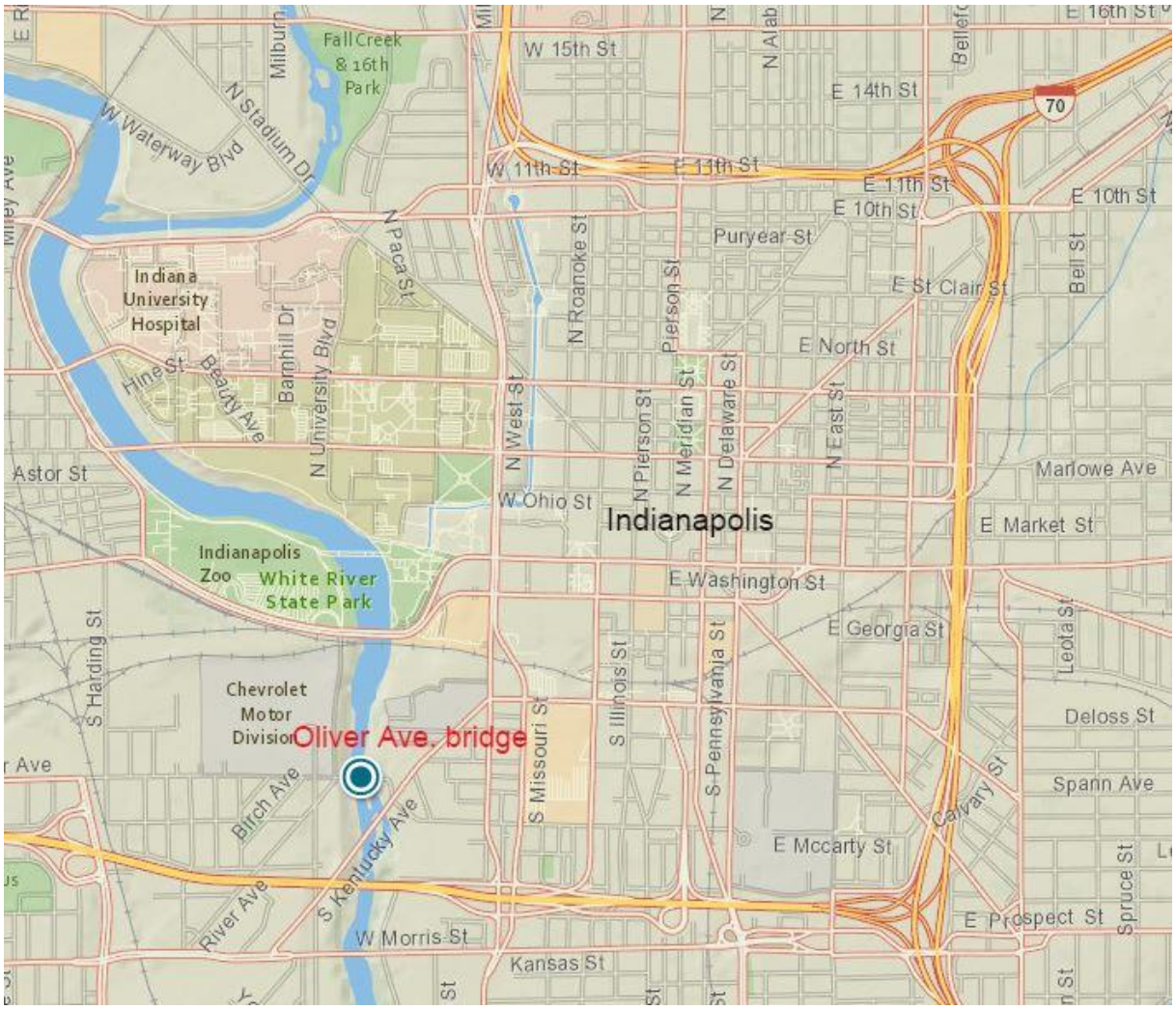


Figure 12. Adapted from Google Maps (2017).

Drainage Basin for Site 3- Indianapolis

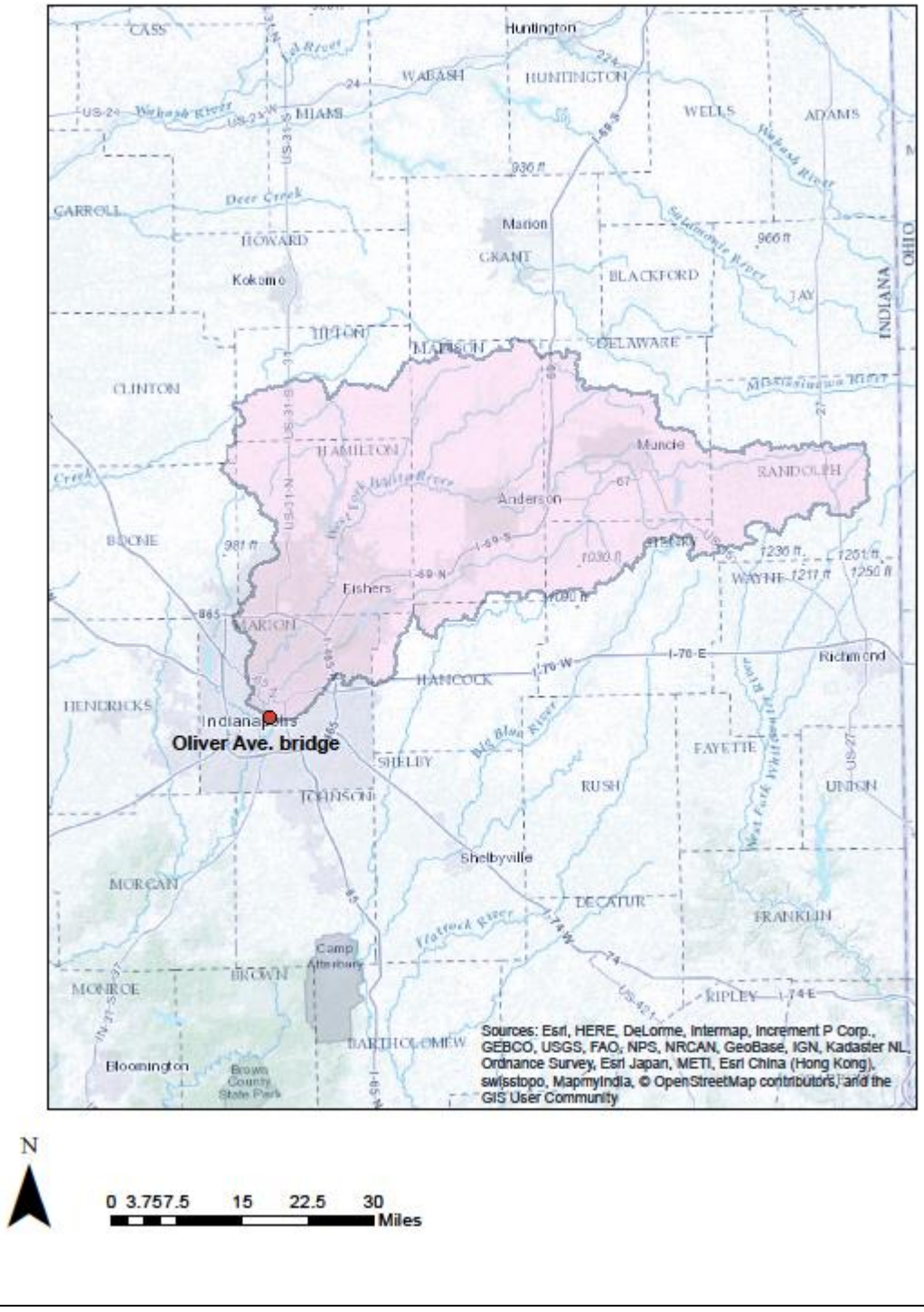


Figure 13

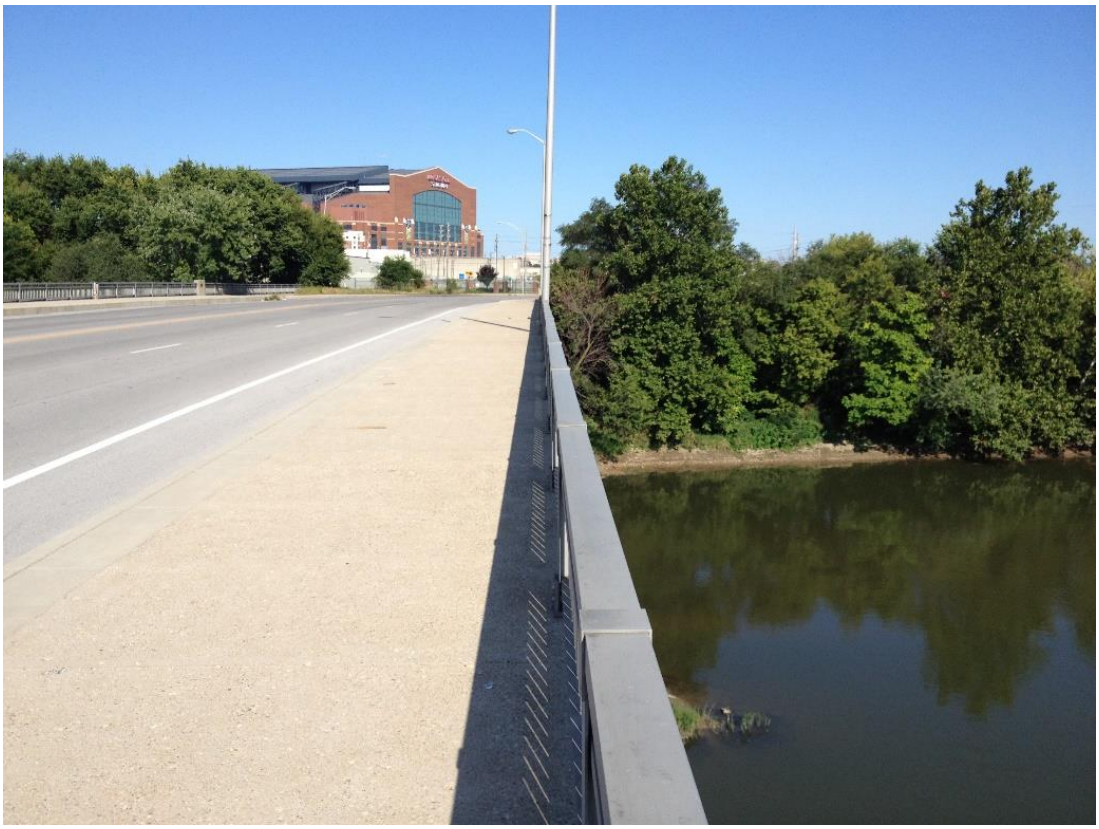
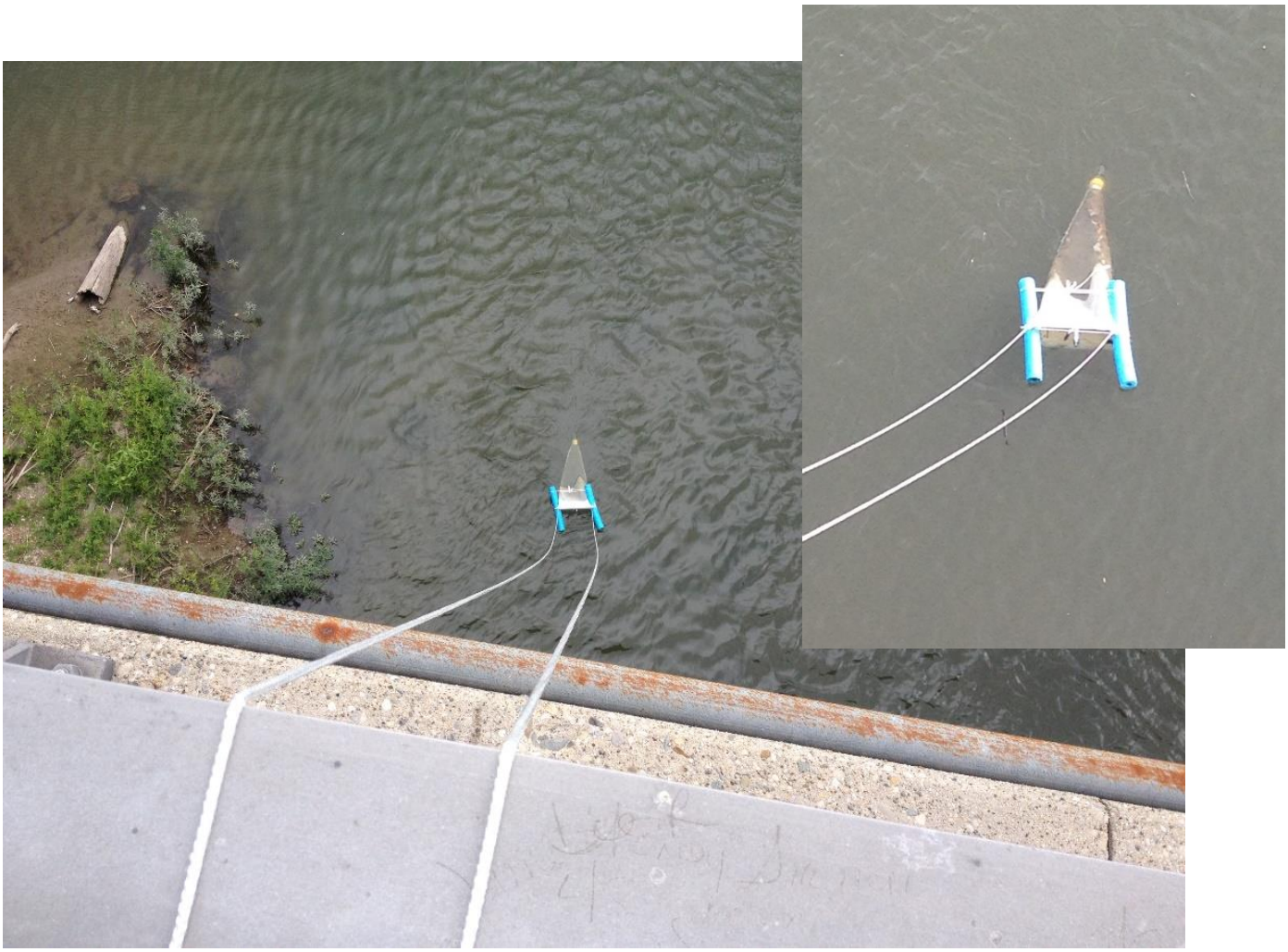


Figure 14

Figure 15



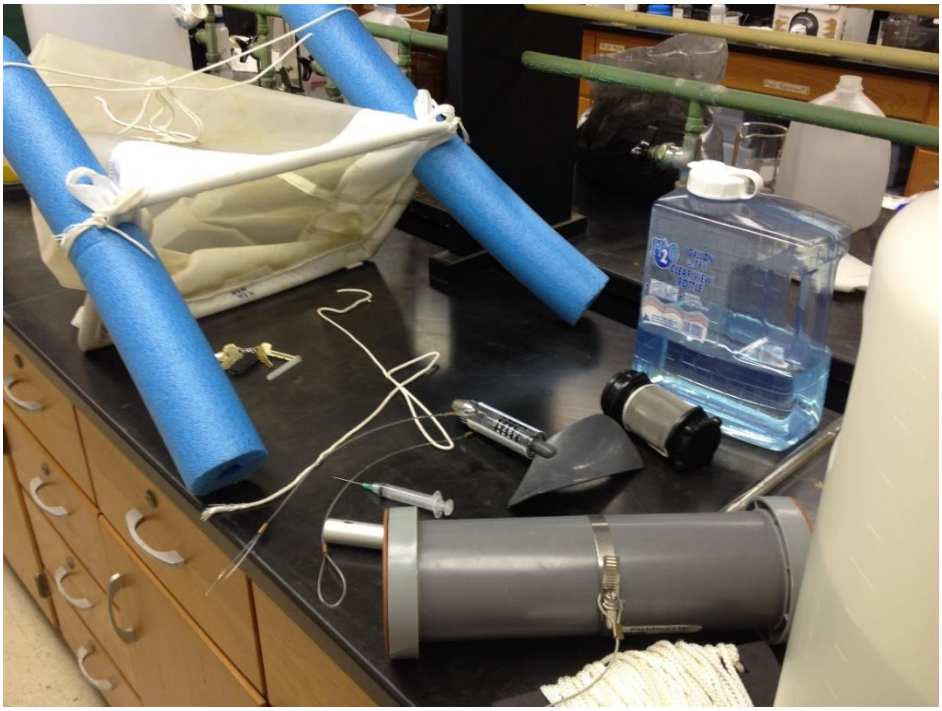


Figure 16

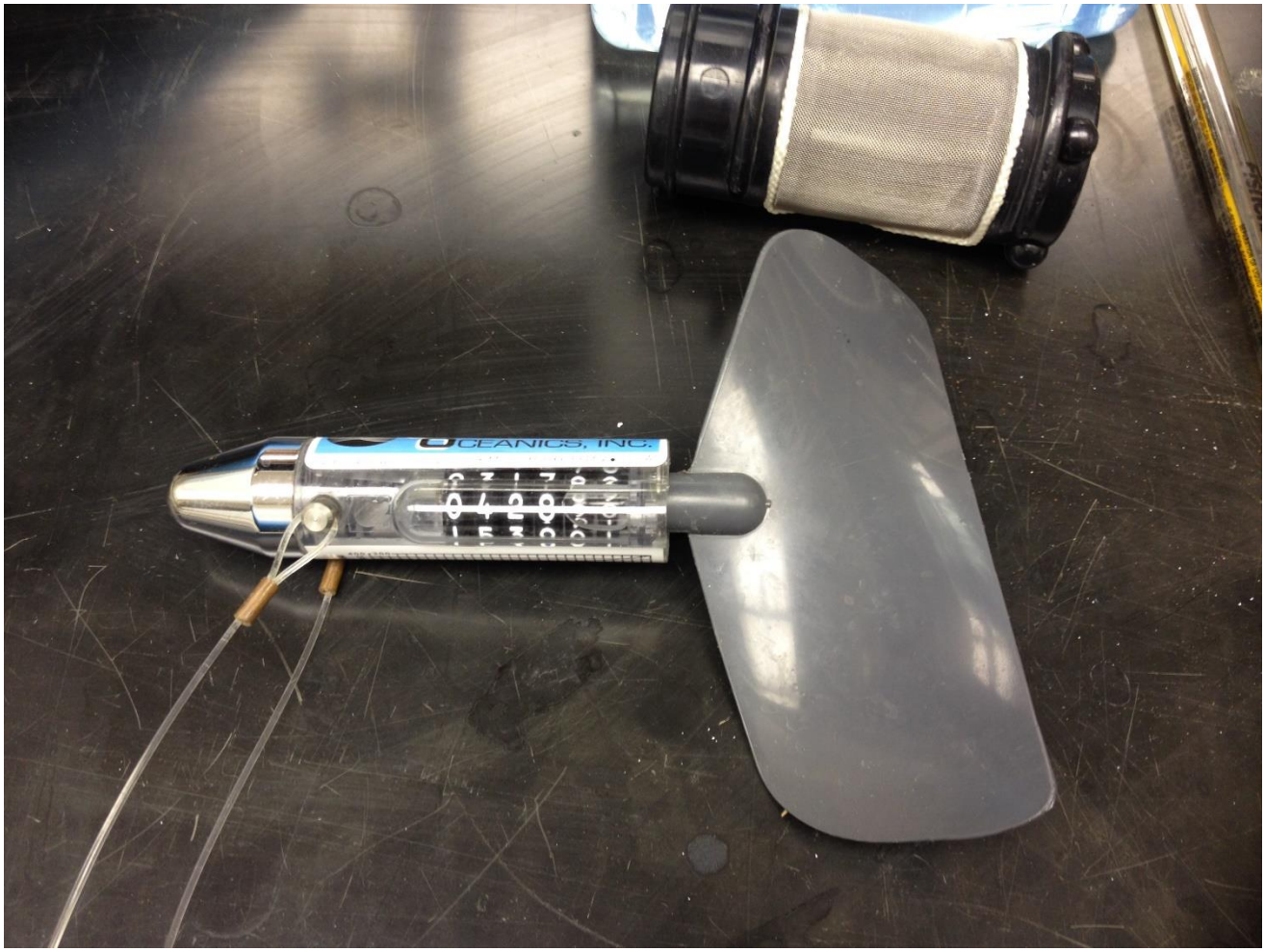


Figure 17

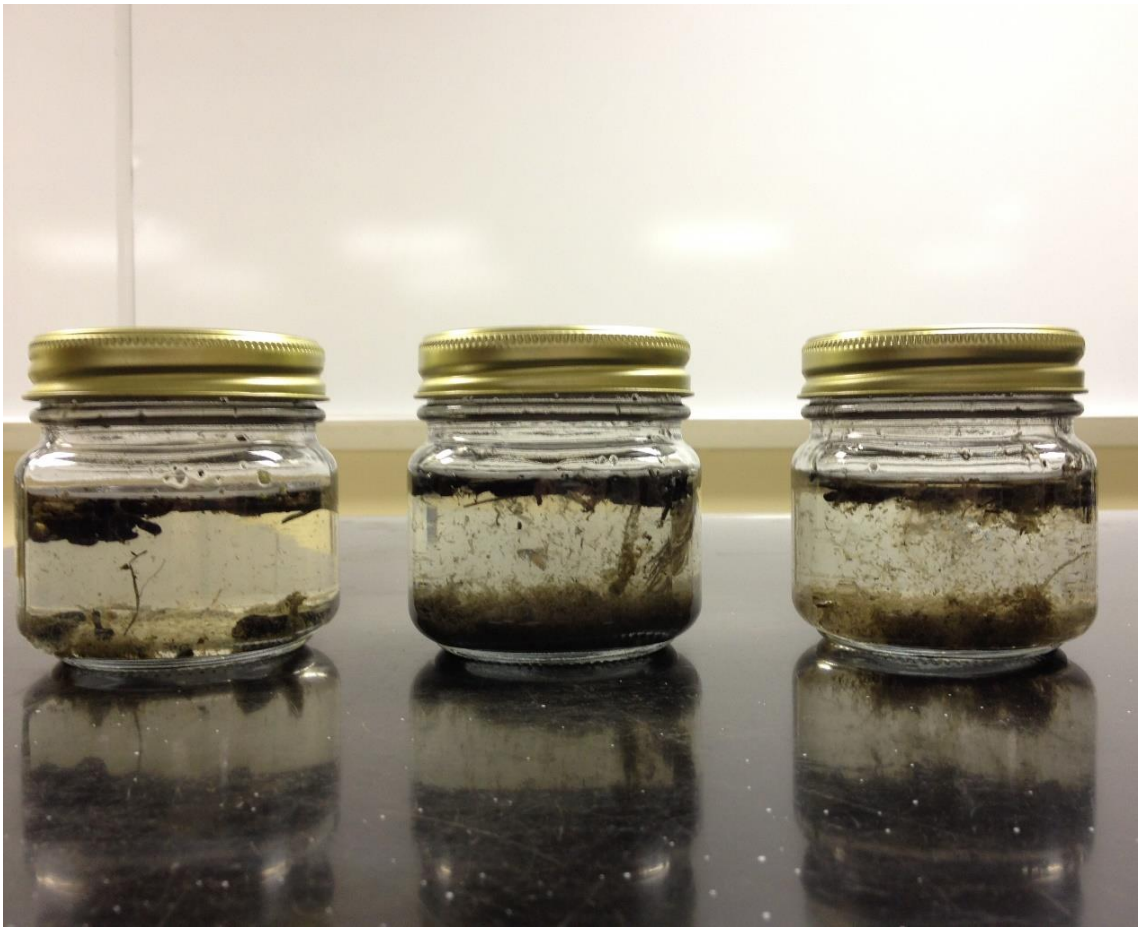


Figure 18

Figure 19

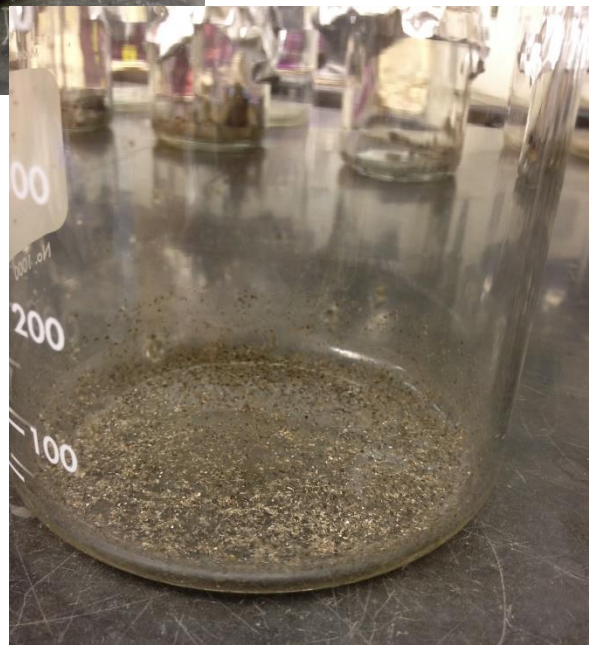
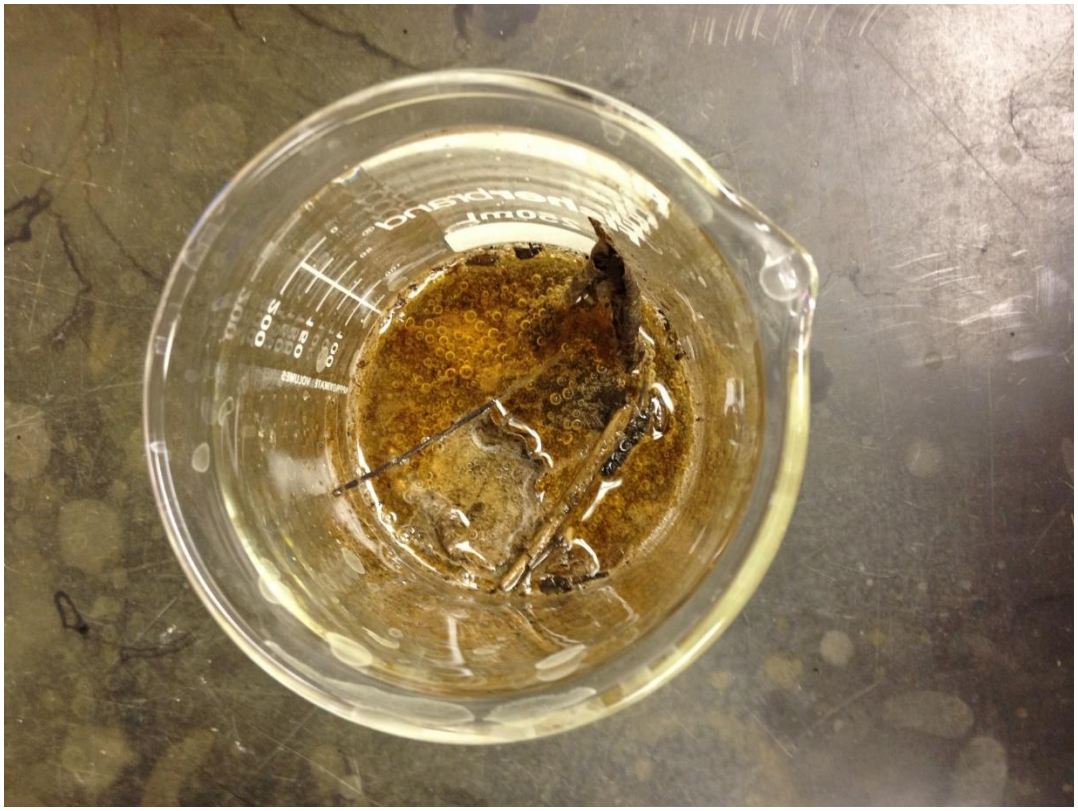


Figure 20




500 mL
±5%
400
300
200
100
HEAT
STIR
SYBRON Thermolyne
nuova 7
STIR-PLATE
TEMPERATURE (x 40°C)
SPEED

Figure 21

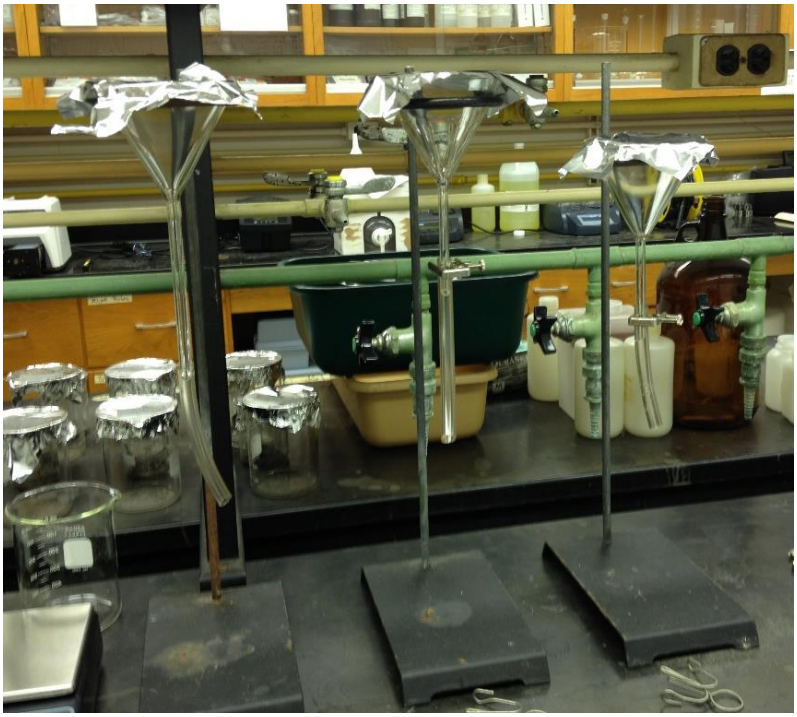


Figure 22

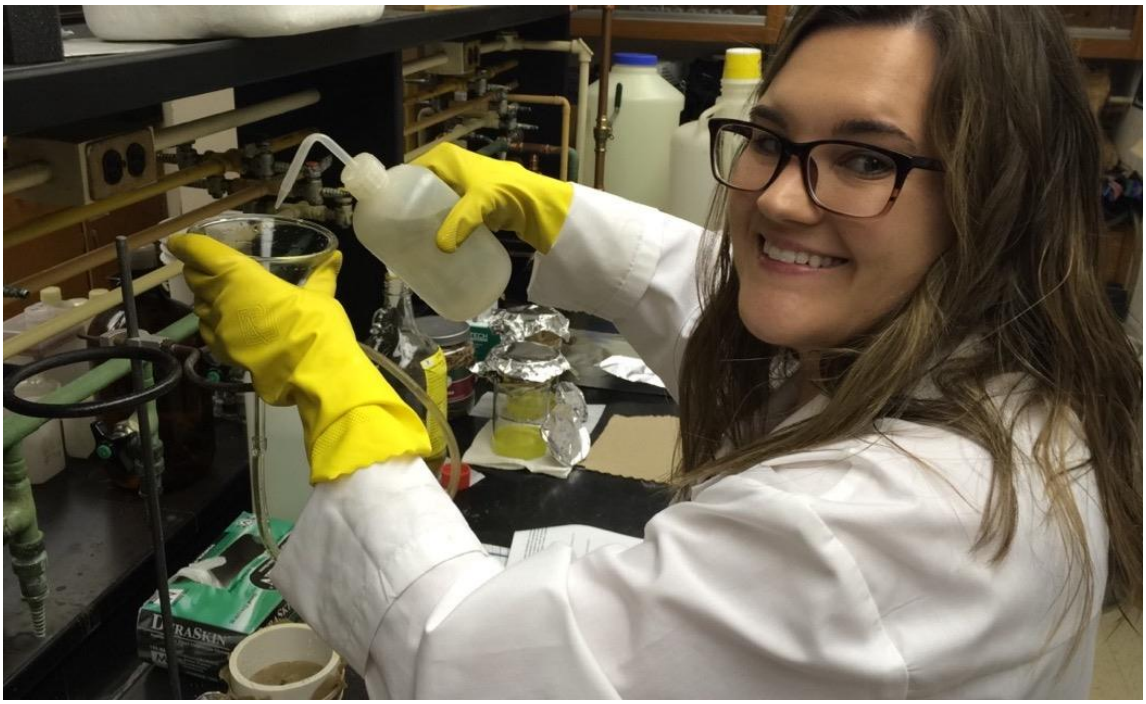


Figure 23

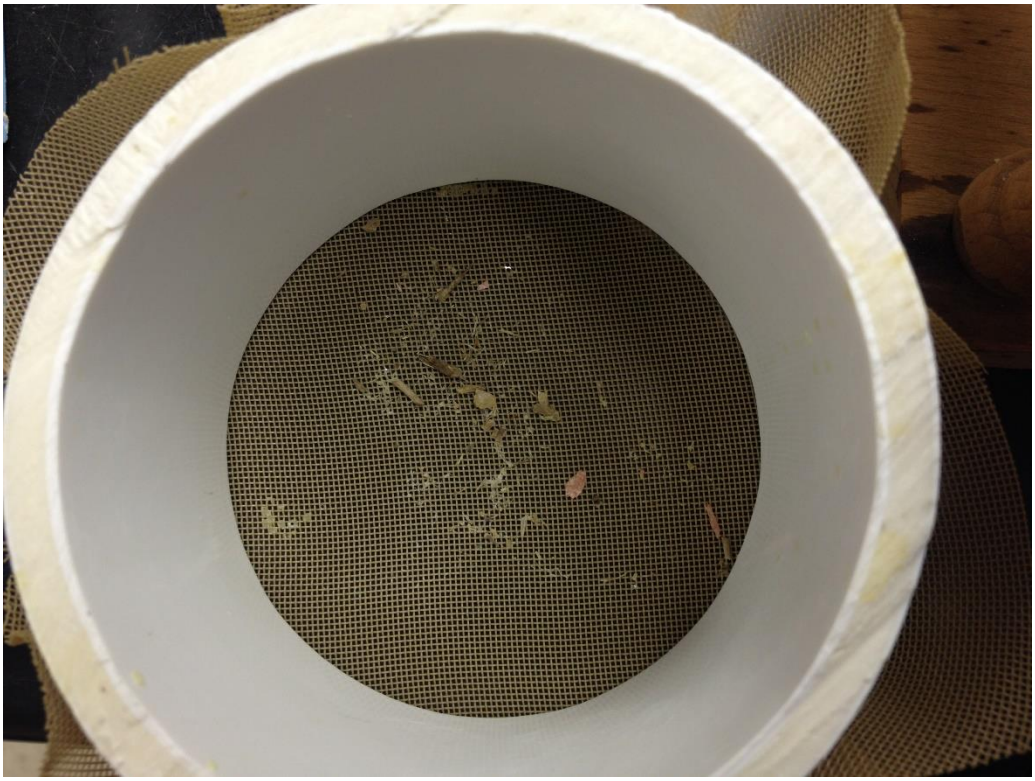
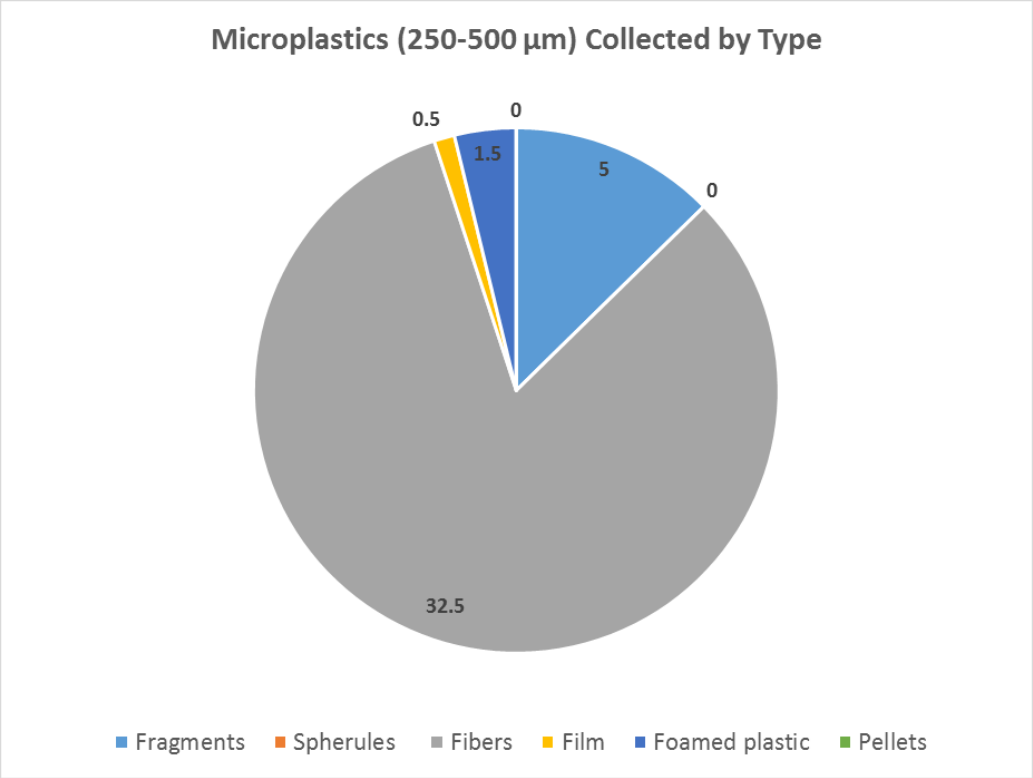


Figure 24

Figure 25

Figure 26


Microplastics (250-500 µm) Collected by Type
0.5
0
1.5
5
0
32.5
Fragments
Spherules
Fibers
Film
Foamed plastic
Pellets

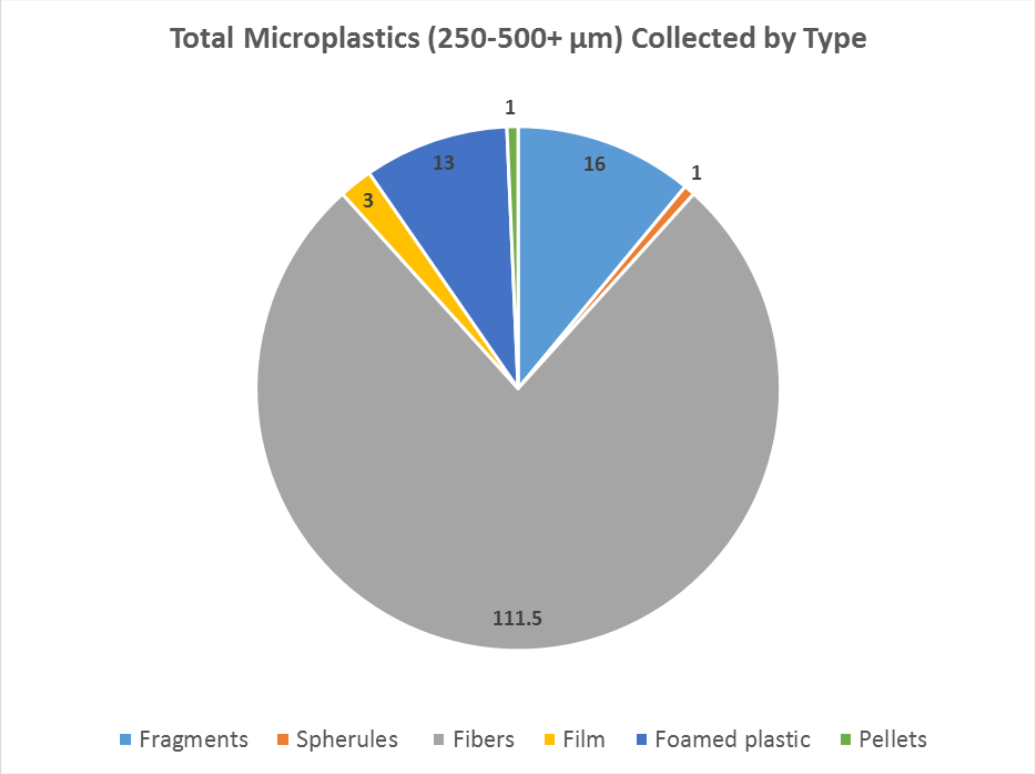
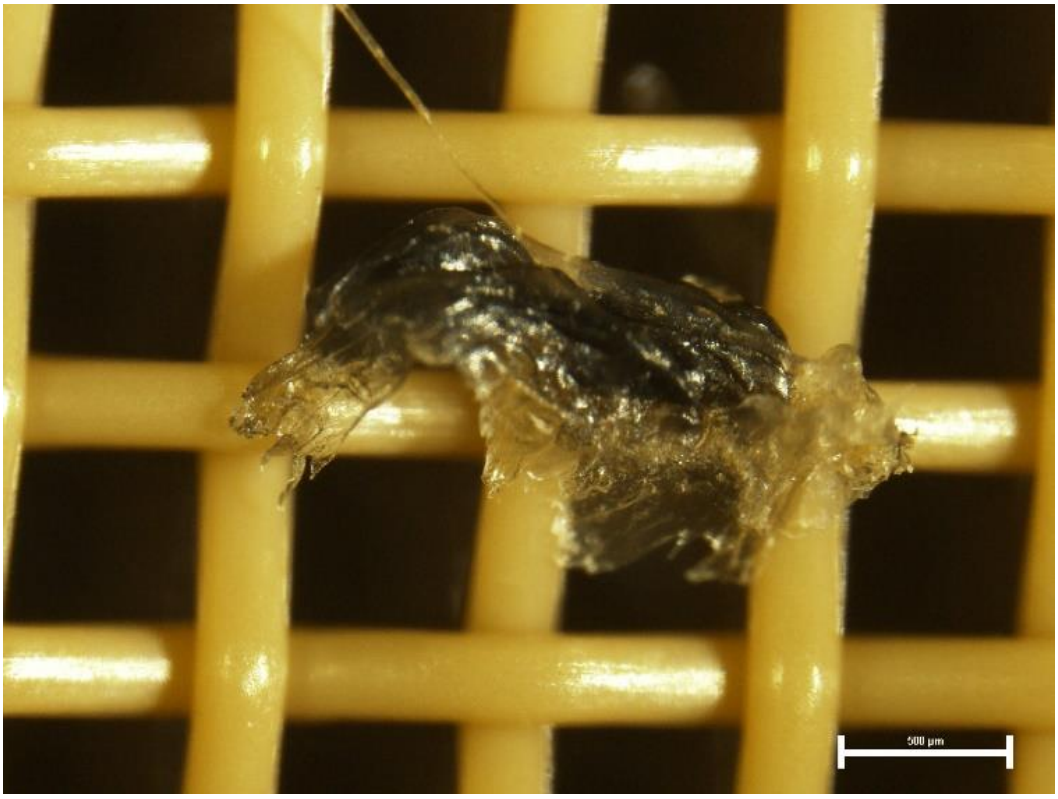
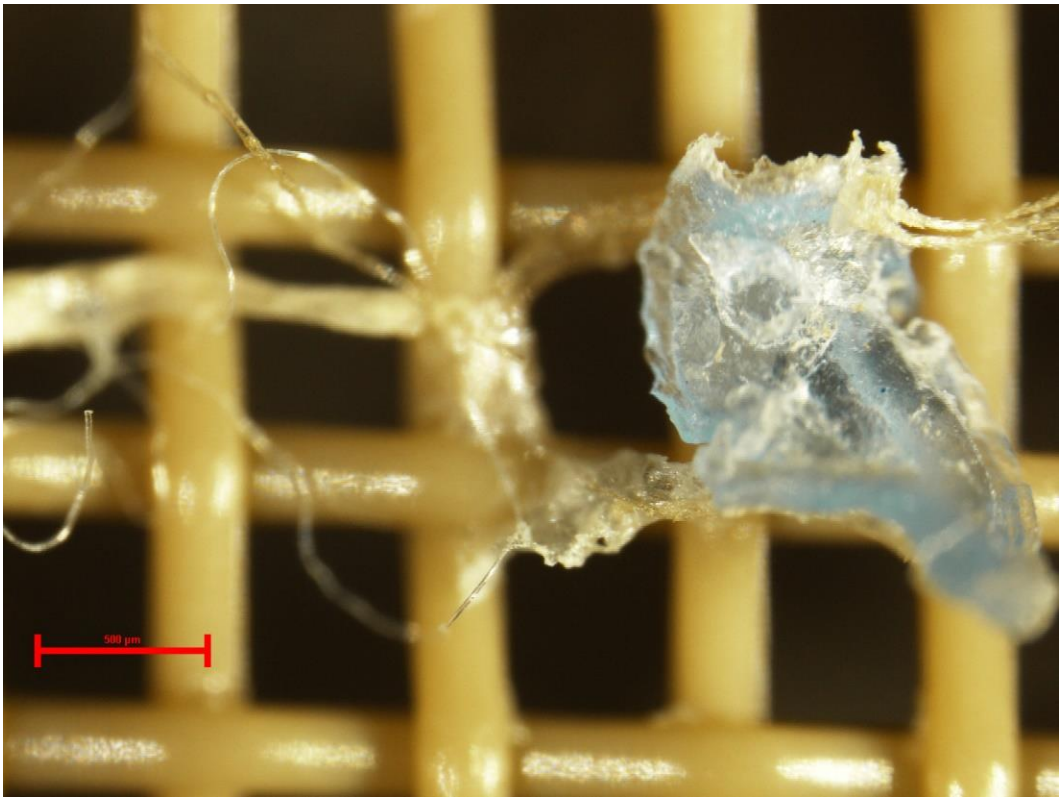
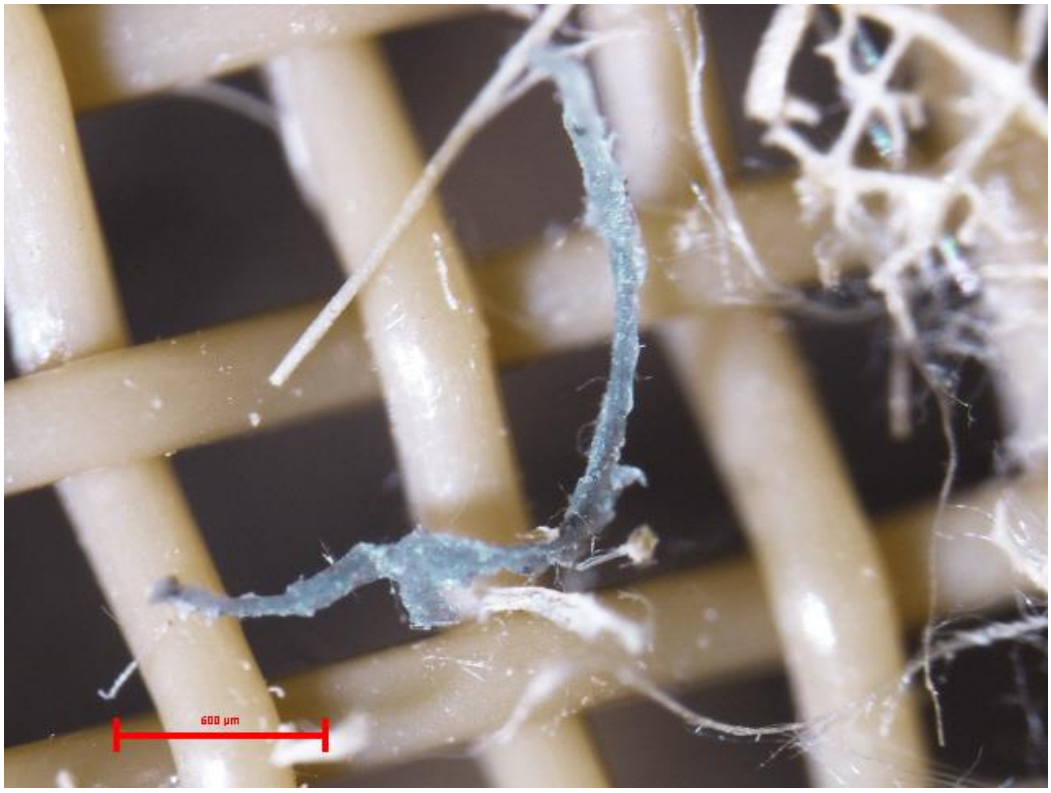
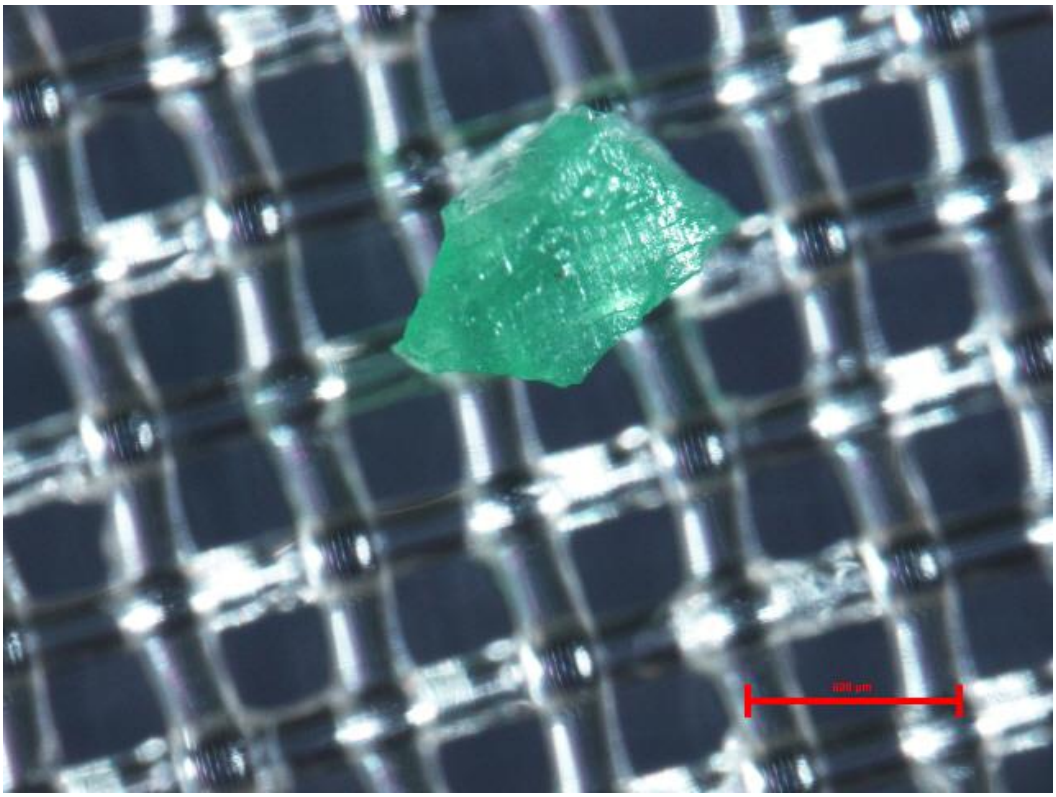

Total Microplastics (250-500+ µm) Collected by Type
1
13
16
1
3
111.5
Fragments
Spherules
Fibers
Film
Foamed plastic
Pellets

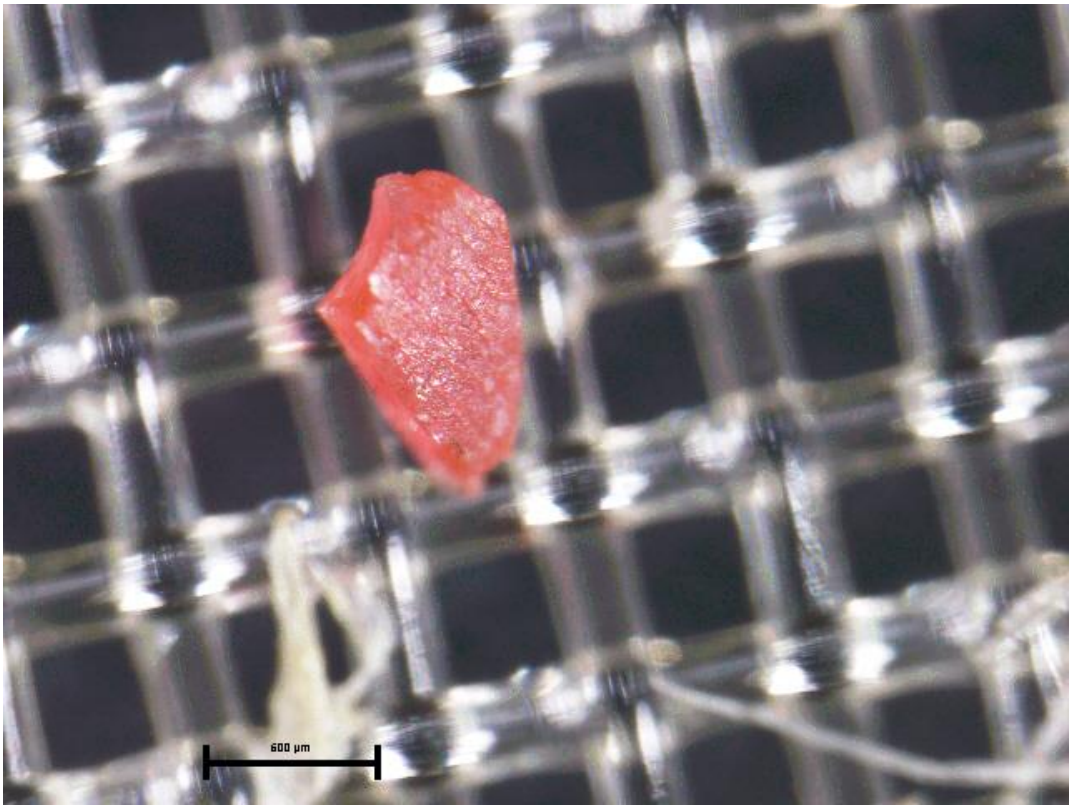
Figure 27

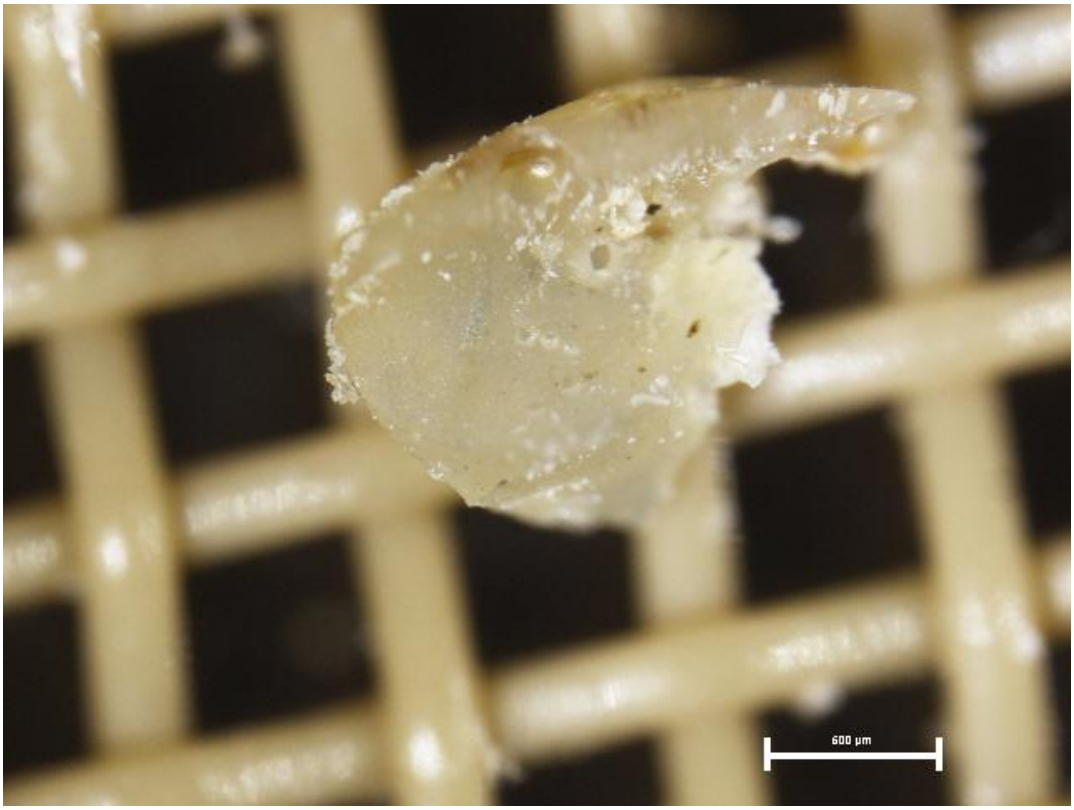

500 µm


500 µm


600 µm


500 µm


600 µm


600 µm

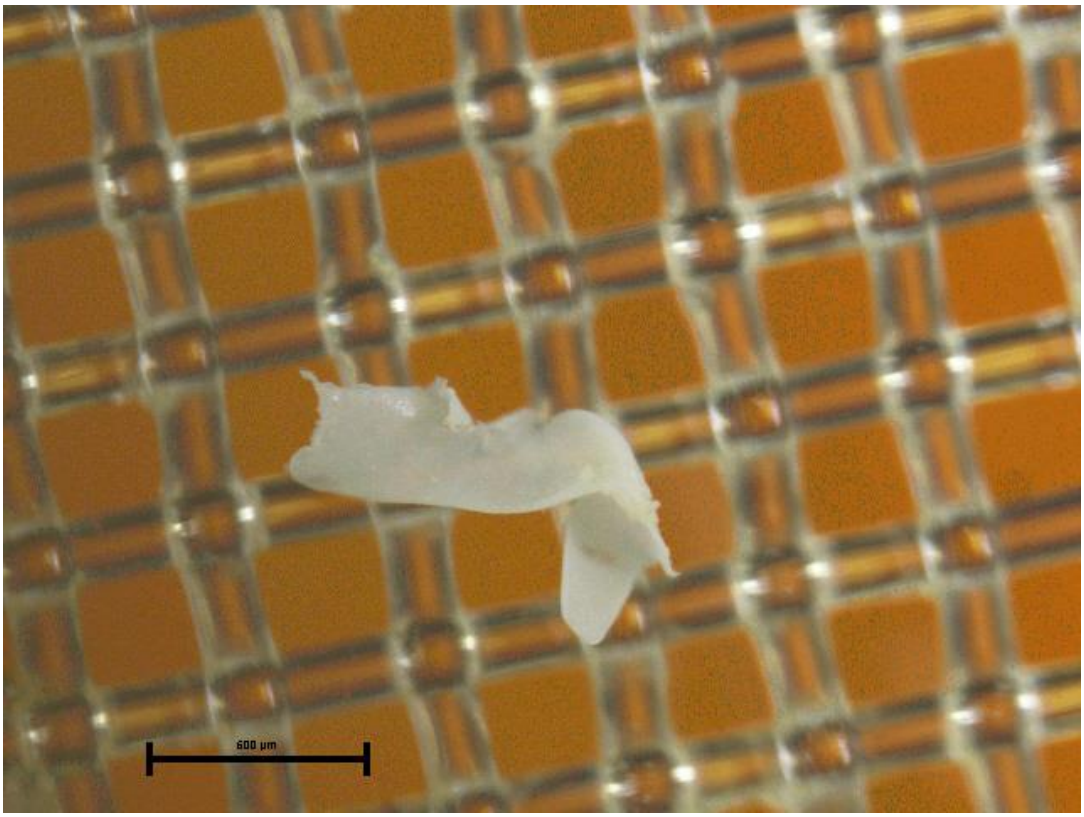
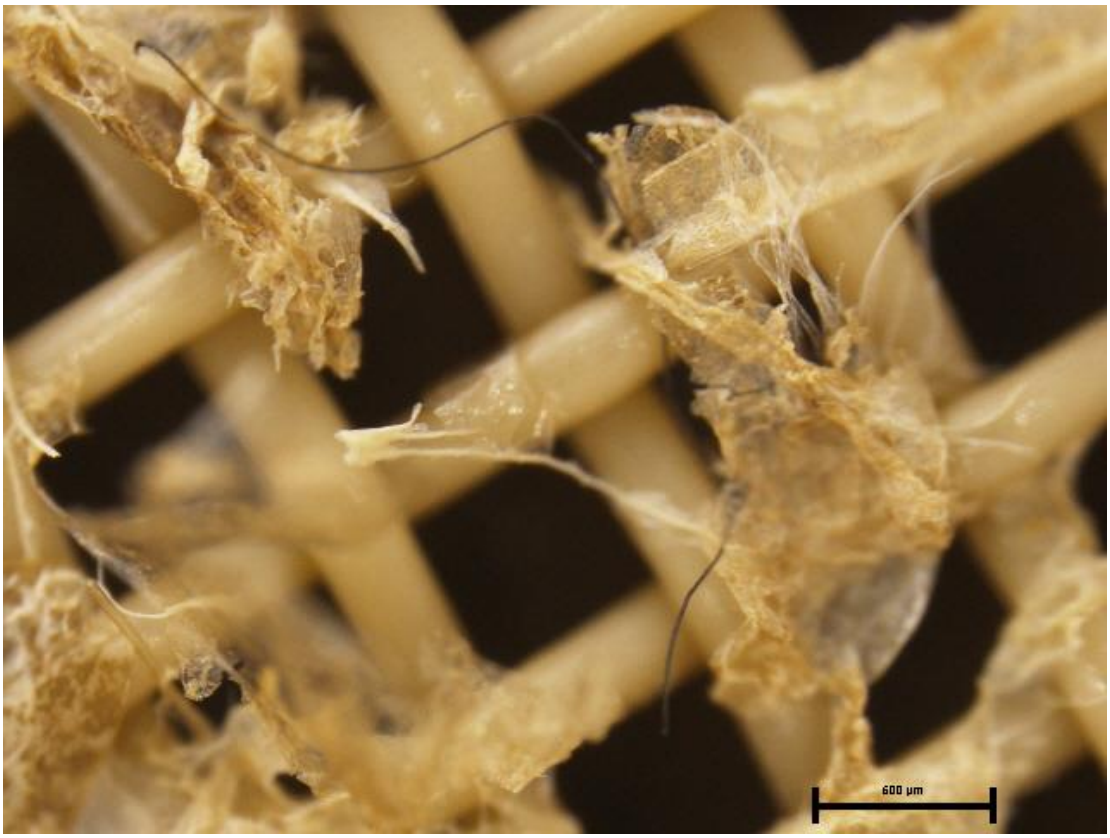
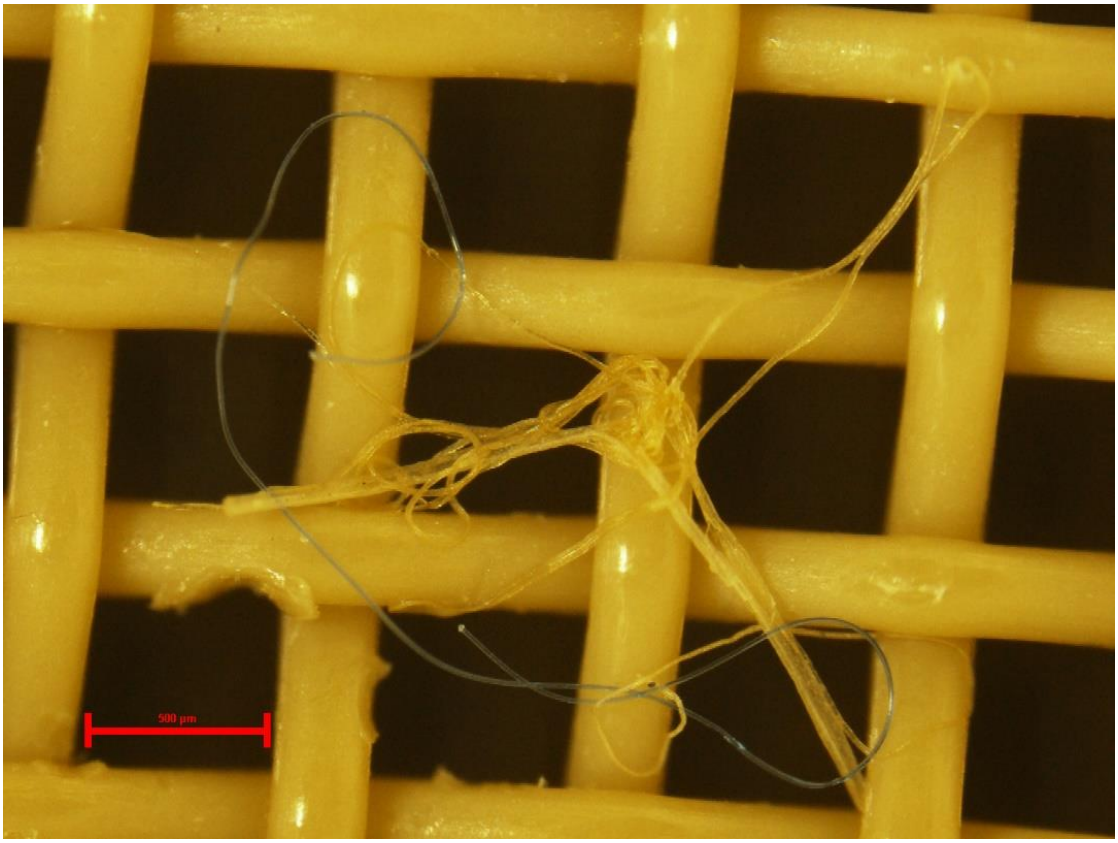
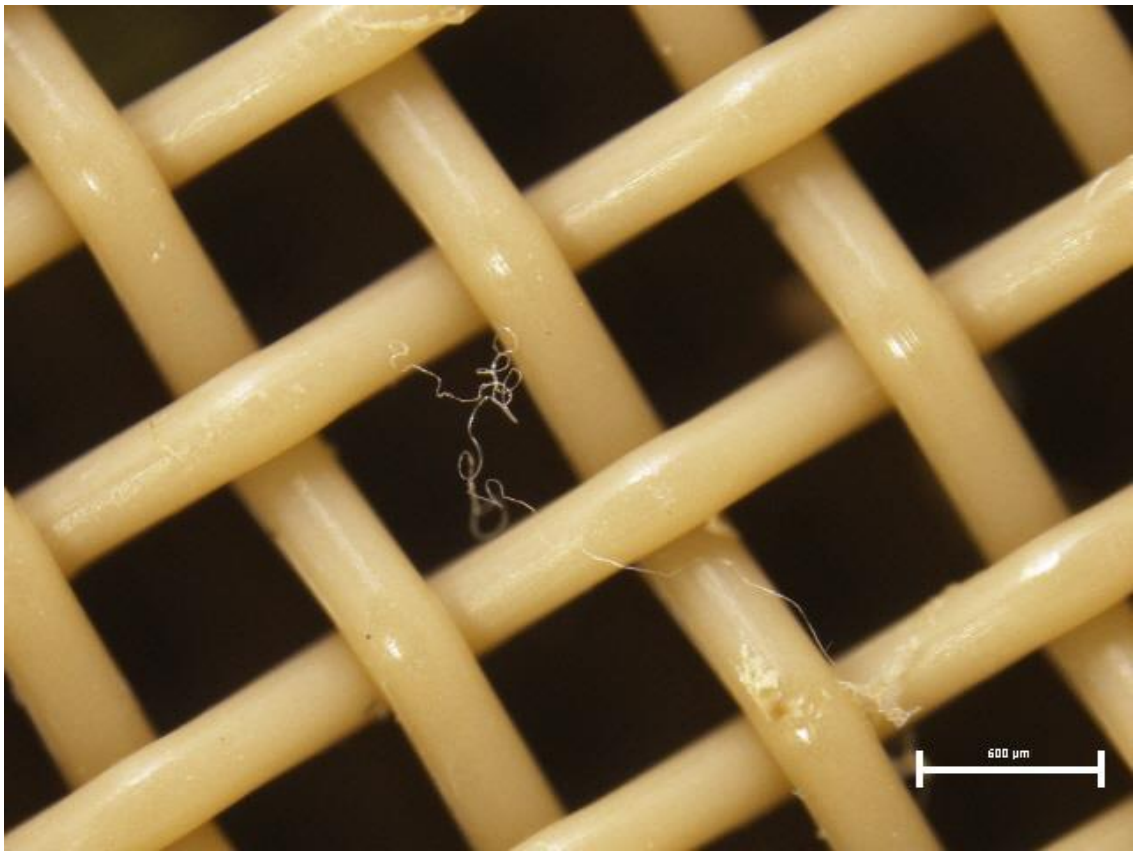
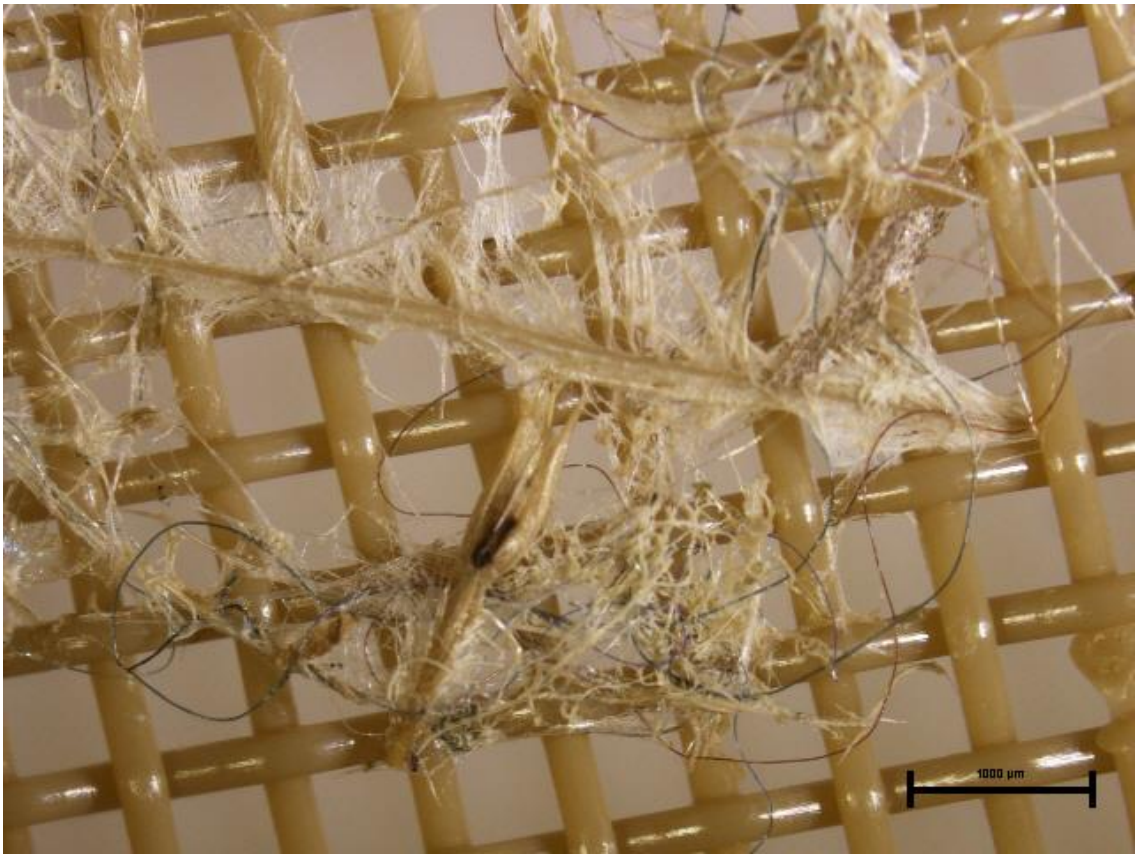


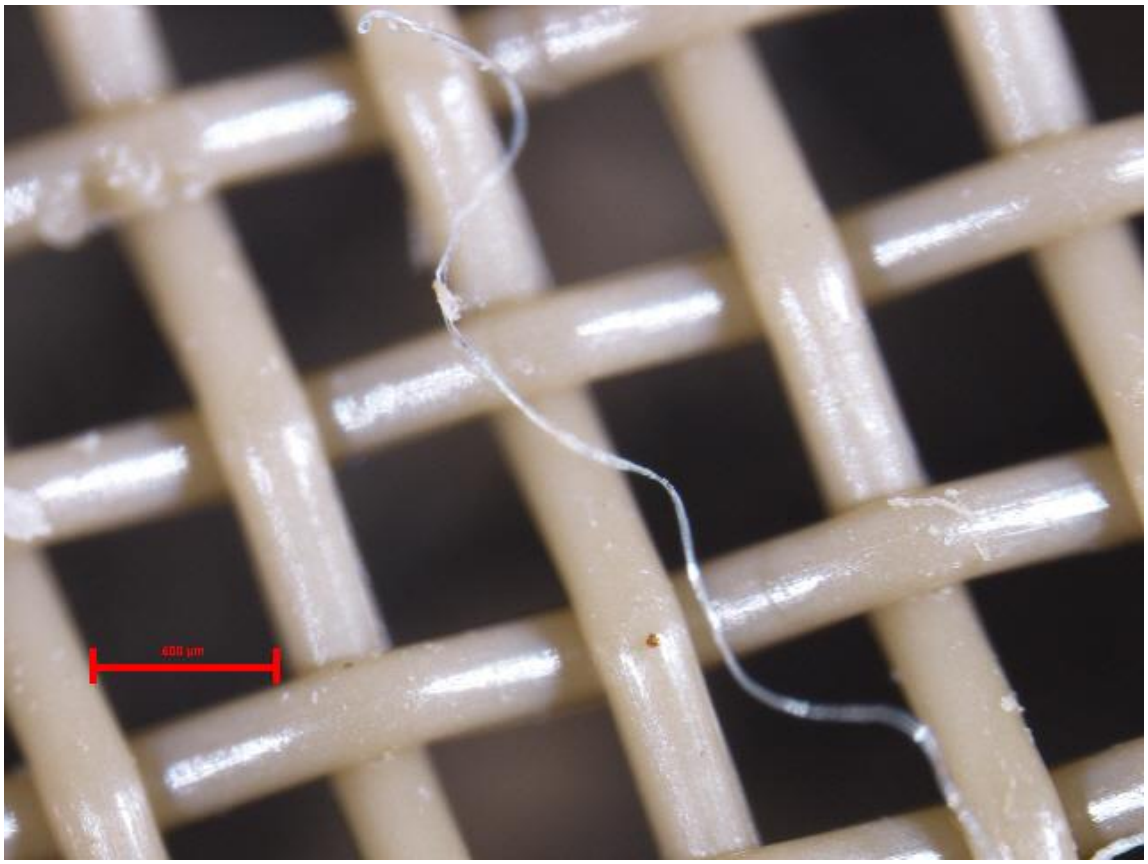
Figure 28

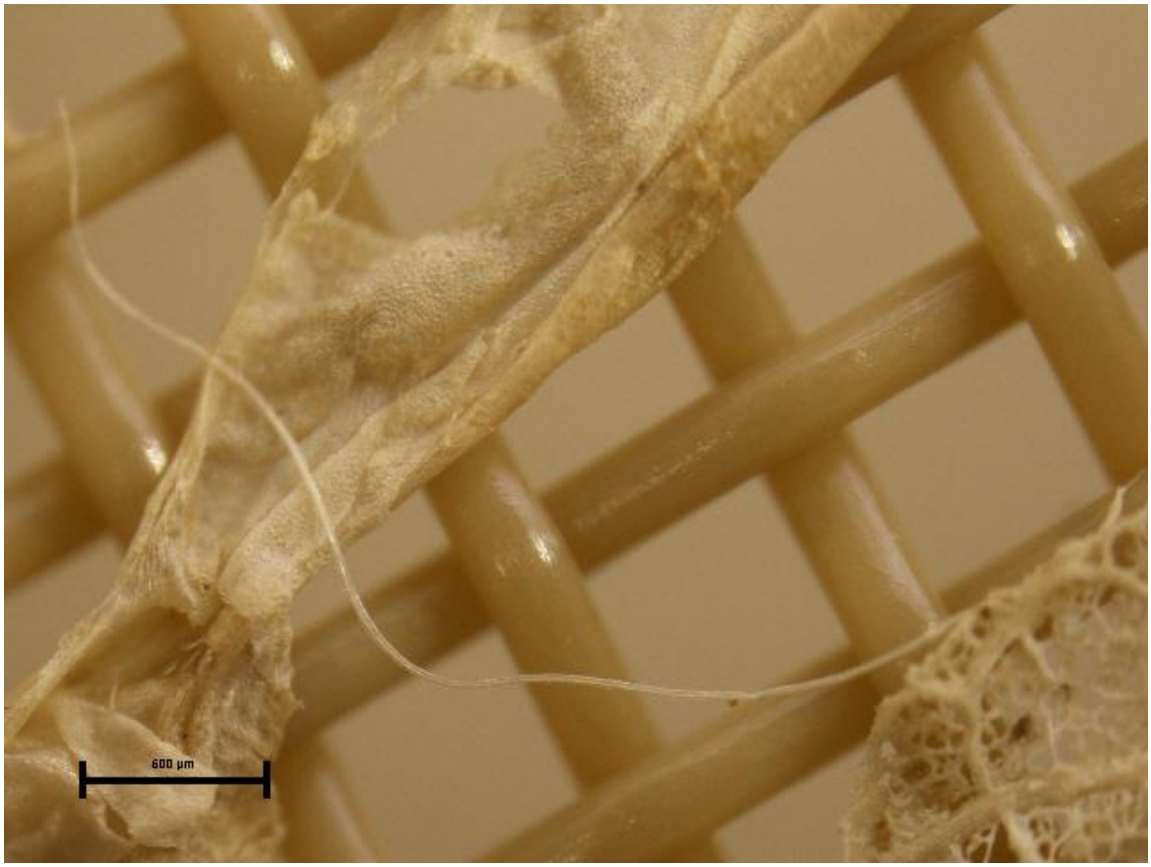

600 µm

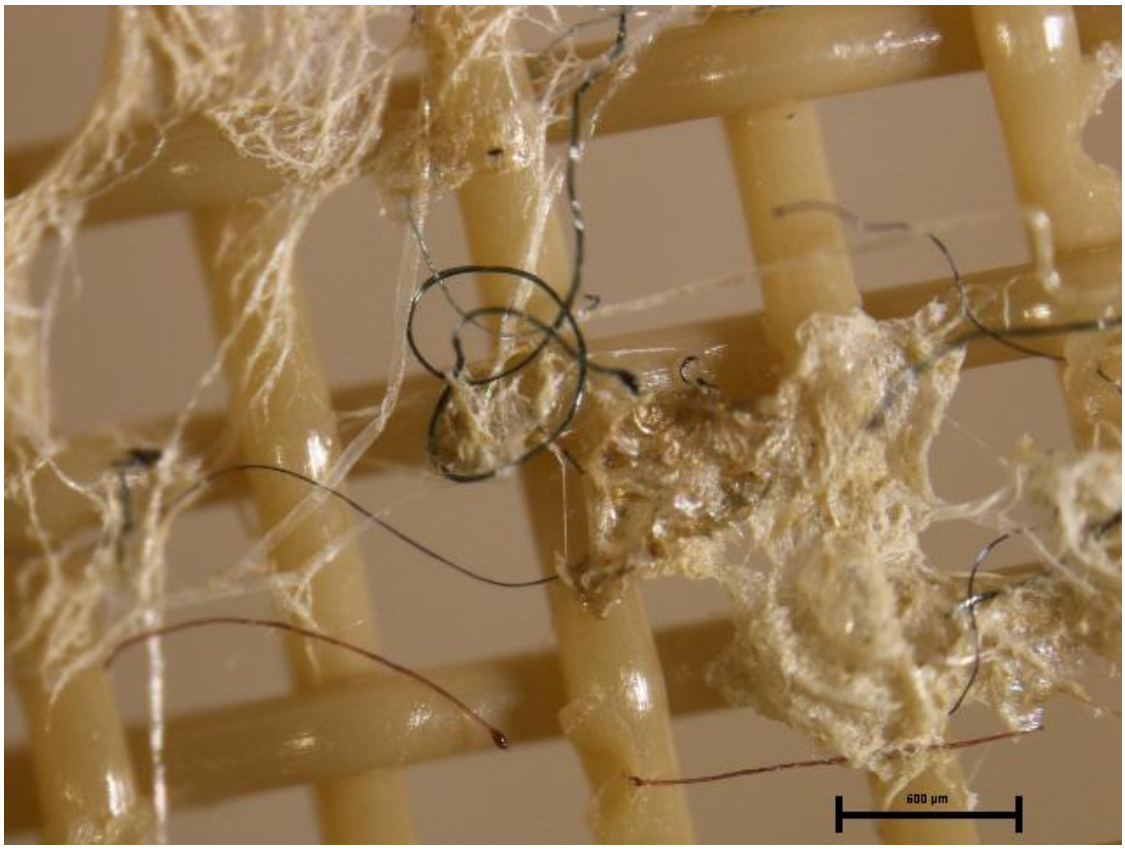

500 µm


600 µm


1000 µm


600 µm


600 µm



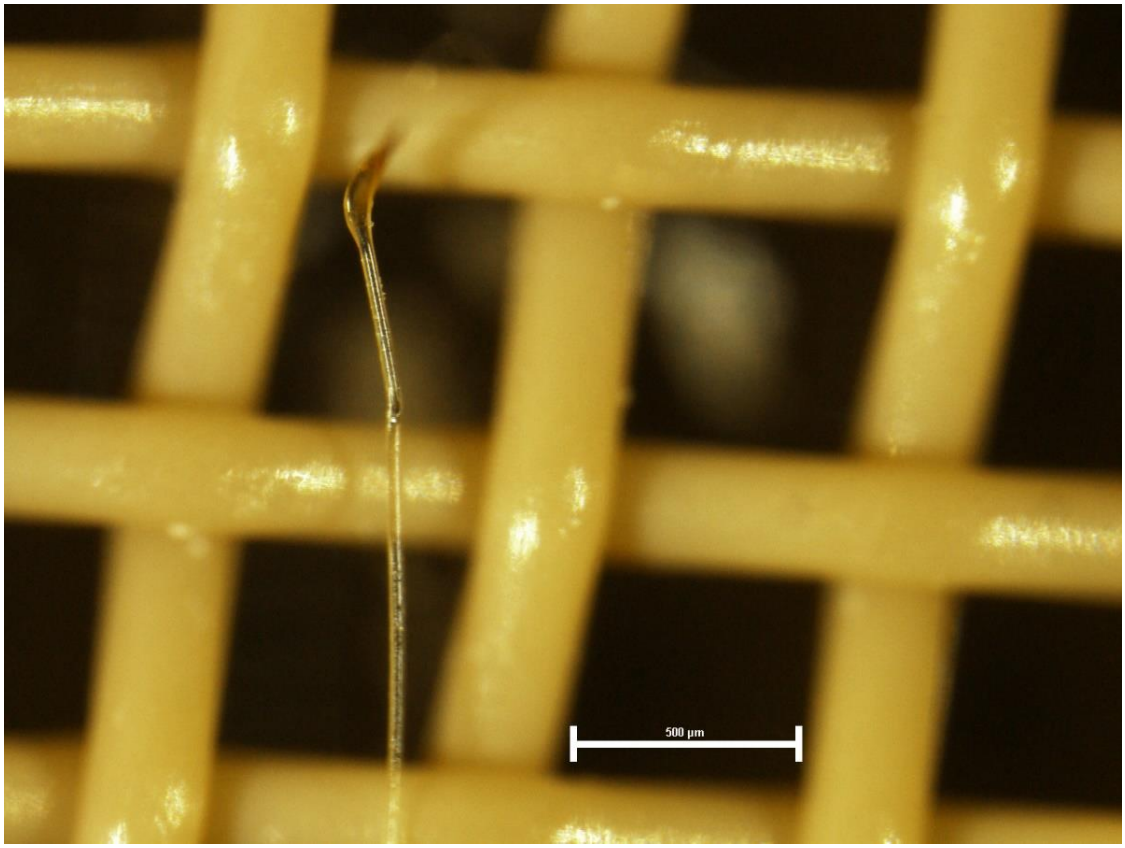


Figure 29

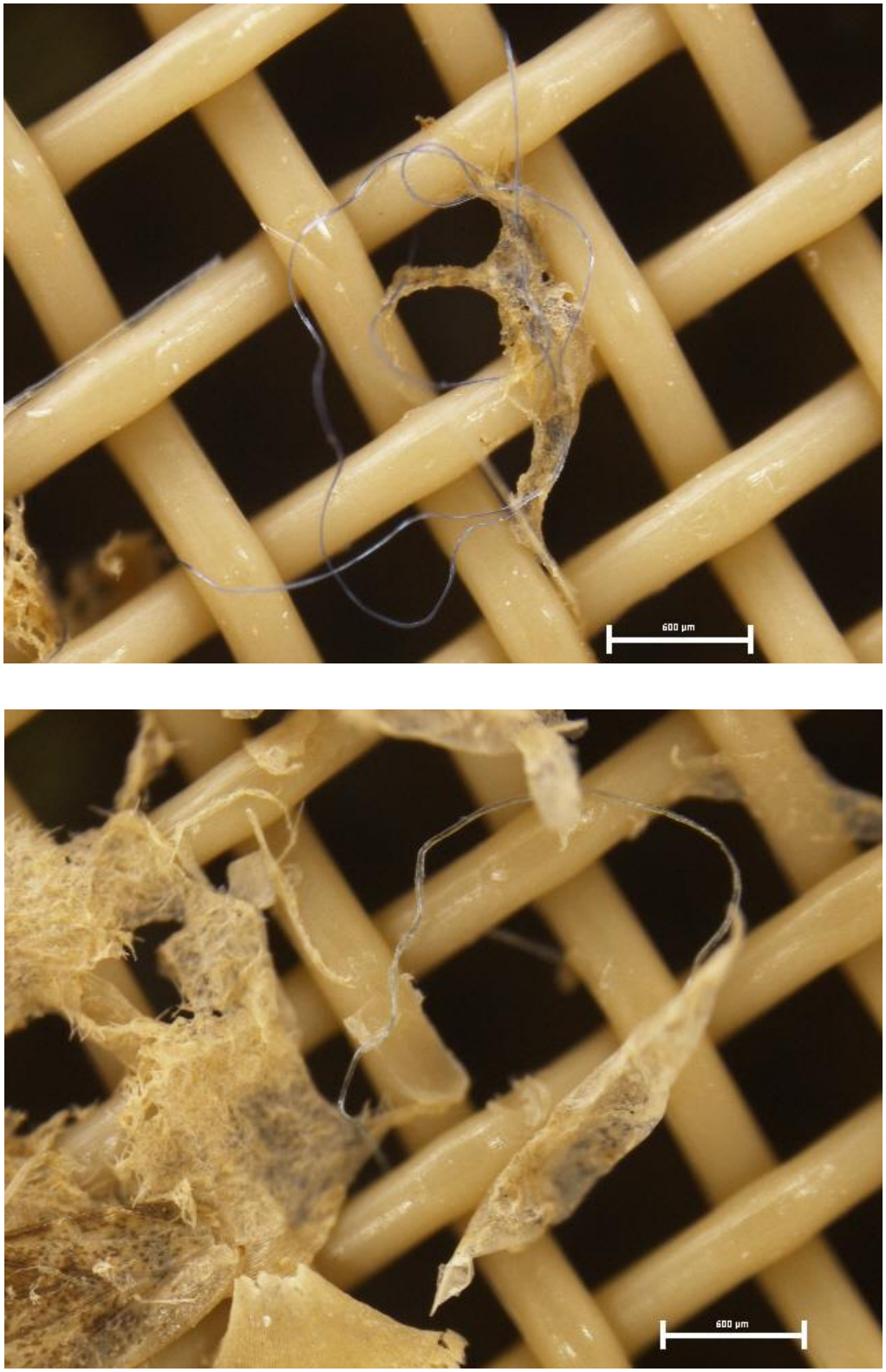


Figure 30

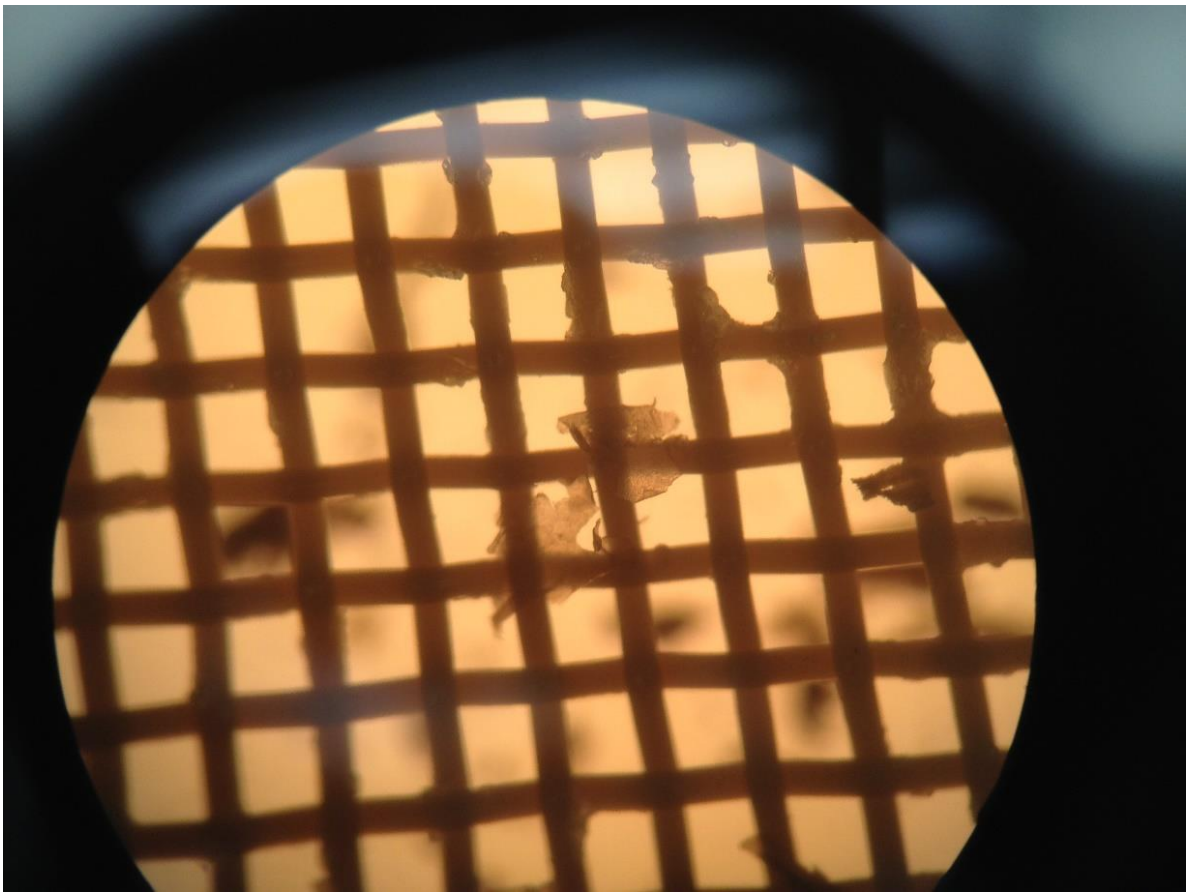


Figure 31

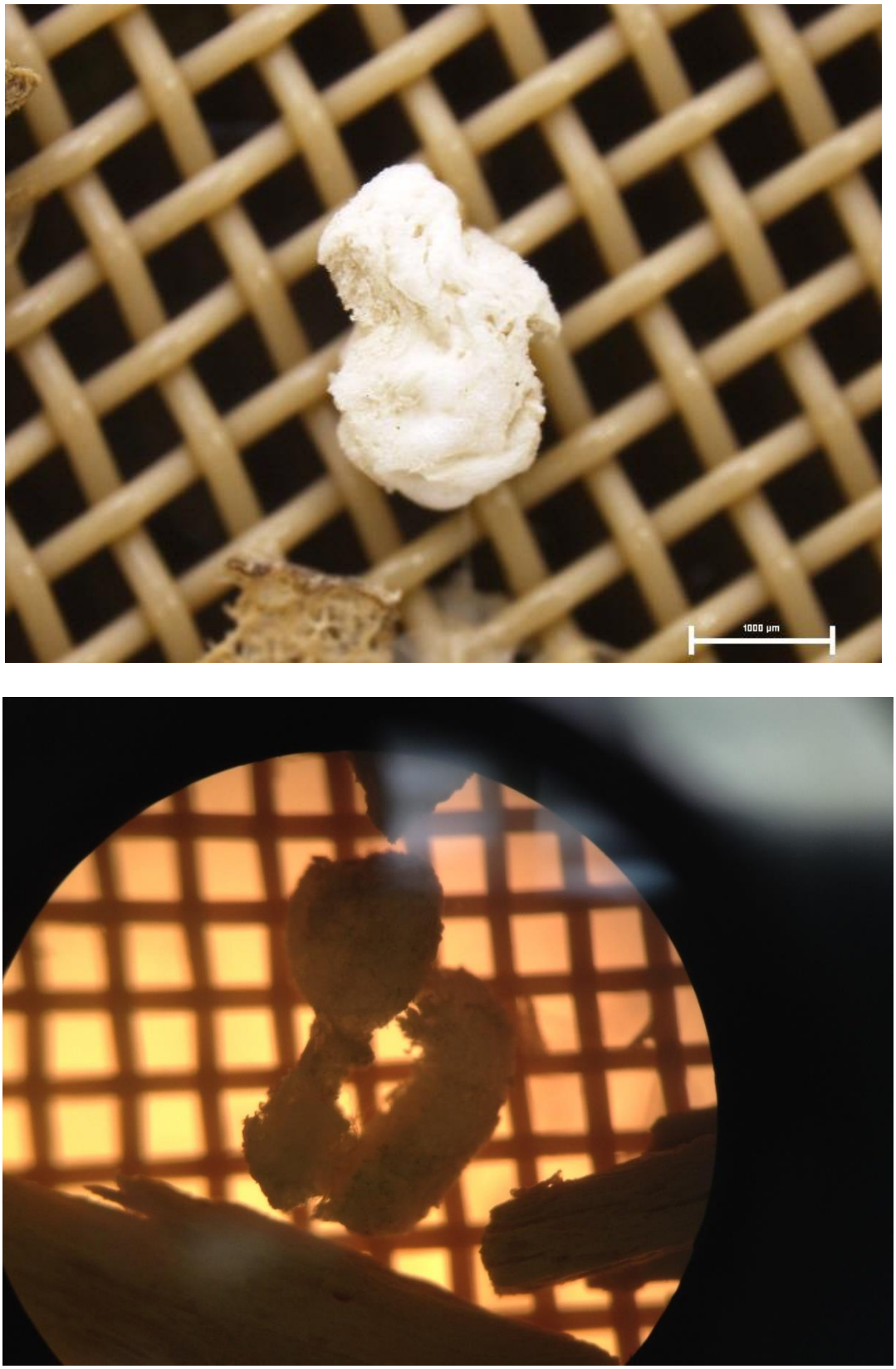


Figure 32

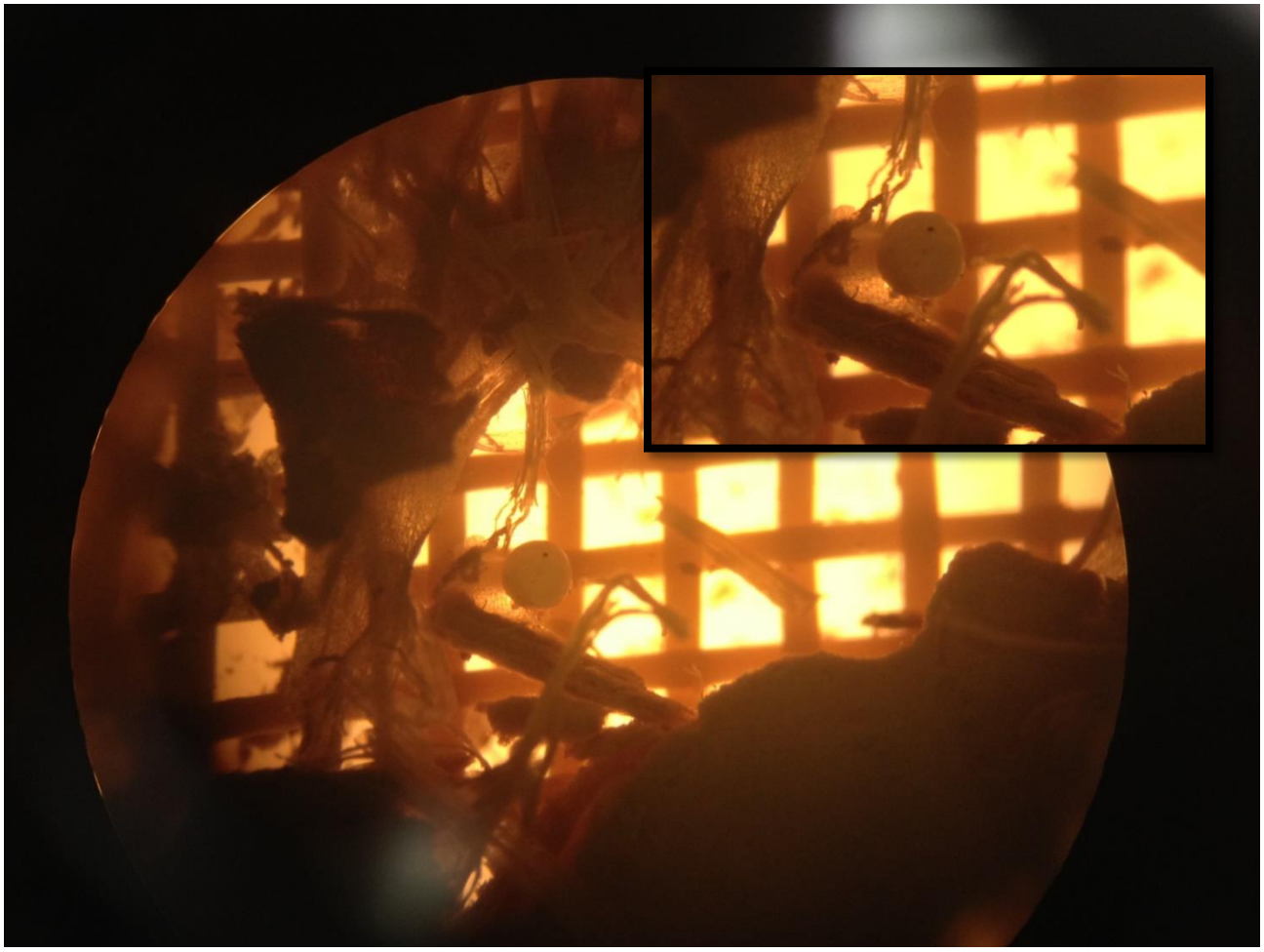


Figure 33

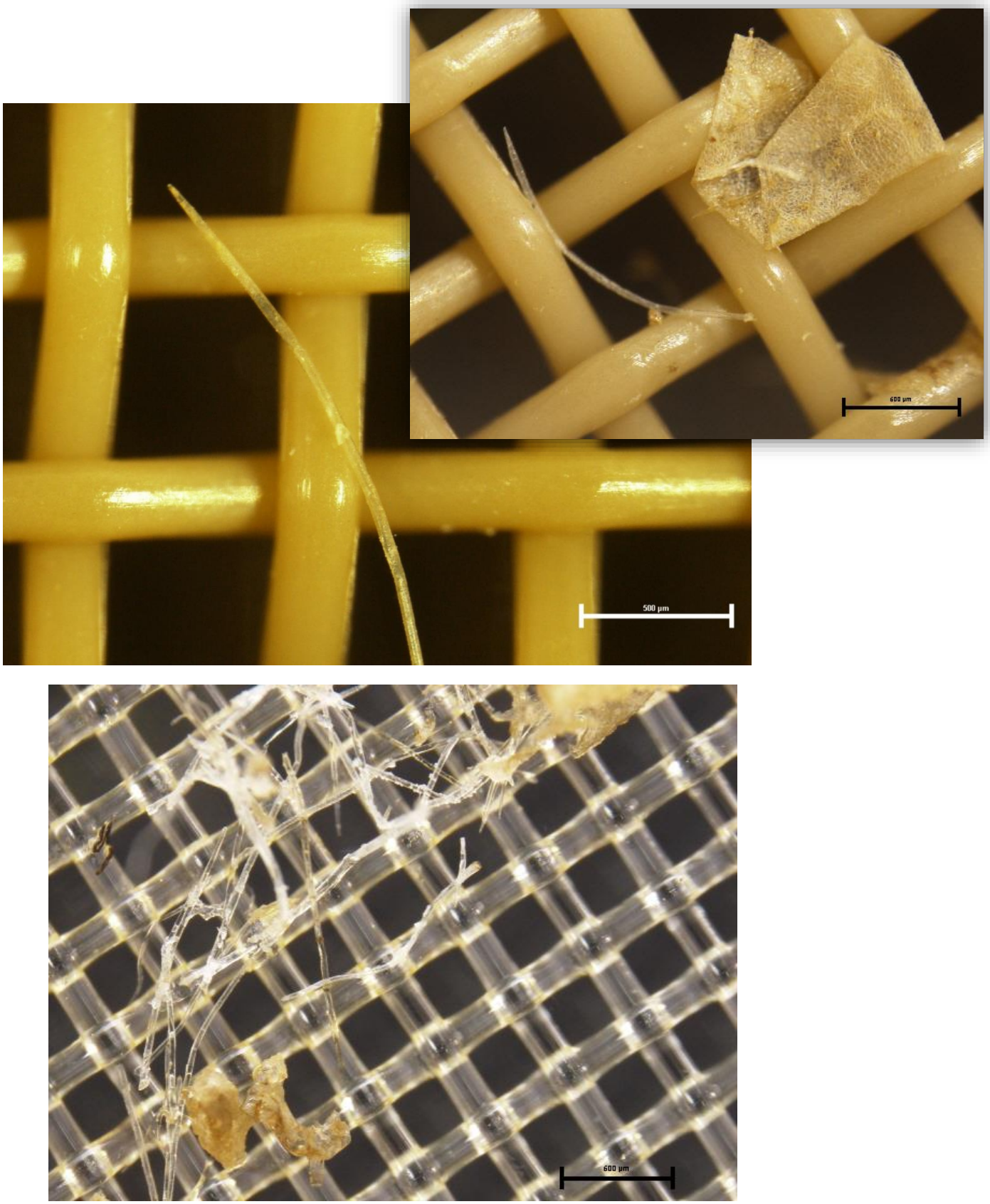


Figure 34

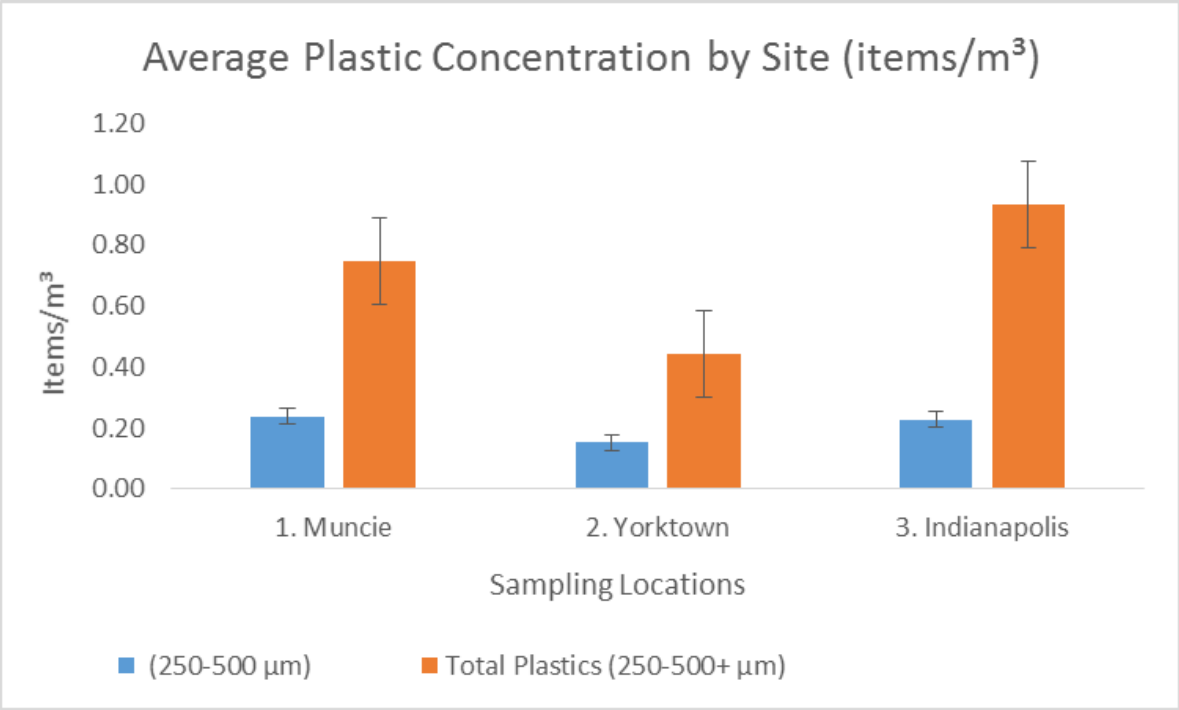

Average Plastic Concentration by Site (items/m³)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Items/m³
1. Muncie
2. Yorktown
3. Indianapolis
Sampling Locations
(250-500 µm)
Total Plastics (250-500+ µm)

Figure 35

APPENDIX

Sample Dates and Times			
Samples using regular rotor			
1AC	Muncie	21-Aug	Morning
1BD	Muncie		
2AC	Yorktown	21-Aug	Afternoon
2BD	Yorktown		
3AC	Indianapolis	21-Aug	Evening
3BD	Indianapolis		
Samples using low-flow rotor			
4AC	Muncie	22-Sep	afternoon
4BD	Muncie		
5AC	Yorktown	26-Sep	afternoon
5BD	Yorktown		
6AC	Indianapolis	26-Sep	evening
6BD	Indianapolis		
7AC	Muncie	8-Oct	10:30 AM
7BD	Muncie		
8AC	Yorktown	9-Oct	5:00 PM
8BD	Yorktown		
9AC	Indianapolis	10-Oct	2:30 PM
9BD	Indianapolis		
10AC	Muncie	22-Oct	2:30 PM
10BD	Muncie		
11AC	Yorktown	23-Oct	7:10 PM
11BD	Yorktown		
12AC	Indianapolis	17-Oct	5:50 PM
12BD	Indianapolis		
13AC	Muncie	5-Nov	3:00 PM
13BD	Muncie		
14AC	Yorktown	9-Nov	4:10 PM
14BD	Yorktown		
15AC	Indianapolis	8-Nov	2:15 PM
15BD	Indianapolis		
**A & B represent pieces captured on the 250 µm sieve			
C & D represent pieces captured on the 500 µm sieve			